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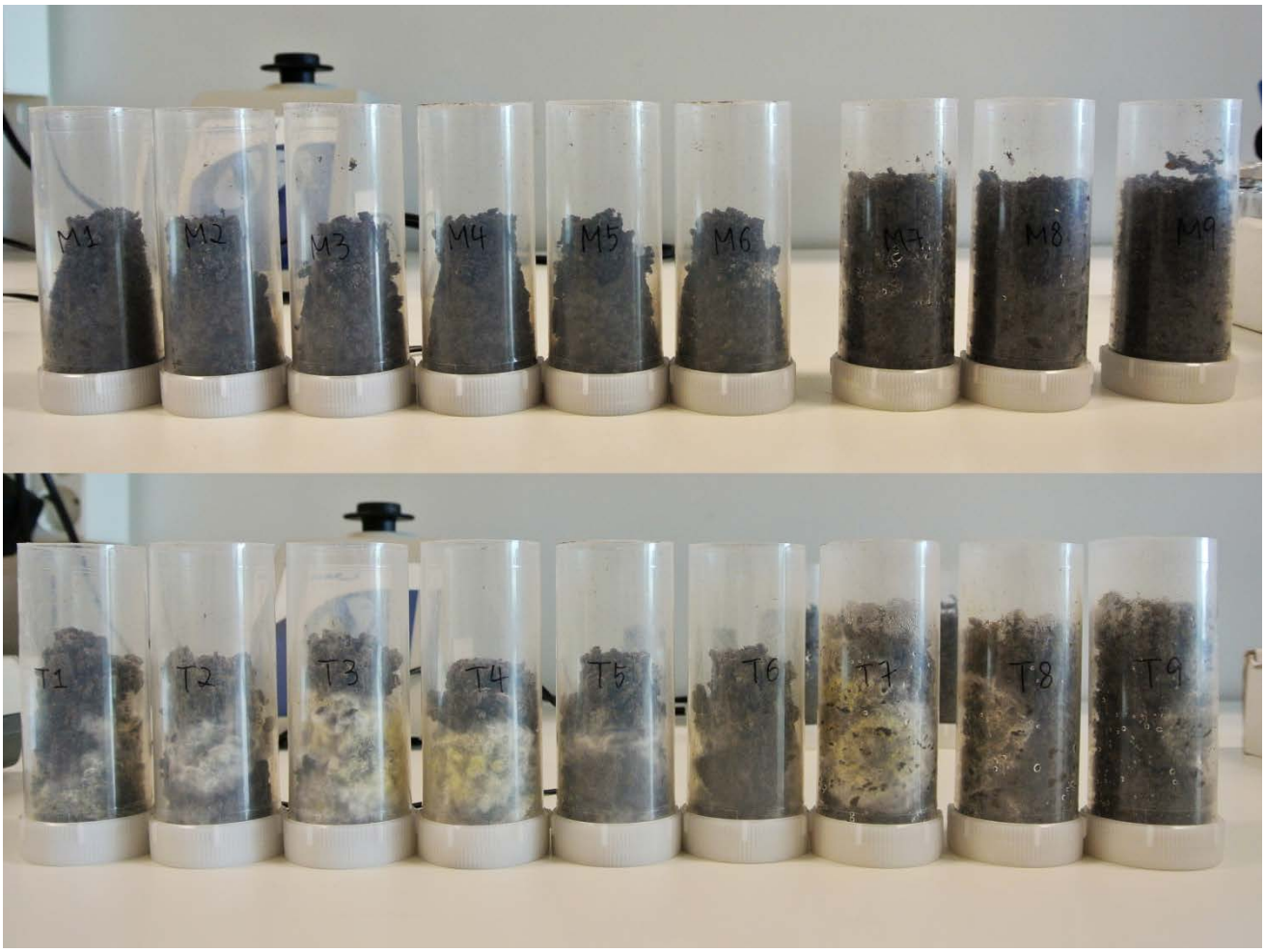

Photo Xi Yang

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Abstract

Sludge produced from wastewater treatment plants is not only rich in nutrients such as nitrogen (N) and phosphorus (P), but also contains toxic organic contaminants and pathogenic organisms which can be potentially hazardous to health of plants, animals and humans. In order to minimize the pathogen contamination in sludge before land application, a minimum of one year of sewage sludge storage has been proposed by Swedish Environmental Protection Agency. Emission of greenhouse gas (GHG) carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) during storage of sludge has been observed, however, studies on the influence of moisture change in the stored sludge caused by natural evaporation and precipitation on the amount of gas emitted are hardly found.

A laboratory study was performed to determine the effect of drying and rewetting of sewage sludge on greenhouse gas emission during the storage phase. Emissions from two types of digested sewage sludge were investigated; mesophilically digested sludge and thermophilically digested sludge. For each substrate, three types of treatments were applied; (1) drying/rewetting (17 days/15 days), (2) drying/rewetting (17 days/15 days) with sludge sampling for chemical analysis at the end of the drying phase and (3) control treatment for which moisture content of sludge during the whole storage phase (32 days) was kept as constant as possible.

Results from the experiment showed that drying slightly reduced the accumulated emission of CO_2 from both substrates and emission of N_2O from thermophilically digested sludge. After the irrigation was applied, a substantial increase in the daily emission of CO_2 from mesophilically digested sludge and daily emission of N_2O from both substrates was observed. Drying and rewetting hardly made any impact on CH_4 emission. Higher carbon emissions from mesophilically digested sludge than that of the thermophilically digested sludge were observed. Reasons to the observations could be due to the difference in: (1) carbon/nitrogen ratio between the two substrates; (2) different moisture content of the two substrates and (3) different digestion treatments applied when producing the sewage sludge.

Preface

This lab-based master thesis was performed at the Department of Energy and Technology at the Swedish University of Agricultural Sciences, Uppsala. The work was carried out under the sincere supervision of Agnes Willén and the examiner of the work was Professor Håkan Jönsson, both at the Department of Energy and Technology, SLU, Uppsala.

First of all, I would like to thank my supervisor Agnes Willén for not only introducing me to this topic but also offering continuous support and valuable advices throughout the whole research process. I also would like to express my deepest gratitude to my examiner Professor Håkan Jönsson, for the guidance and inspirations provided for better improving the report. Special thanks goes to the Swedish Research Council Formas, the Development Fund of the Swedish Water Association (Svenskt Vatten Utveckling) and Vinnova for the financial support.

Surely, I would not have accomplished this challenging and interesting project without the help and support of others. I would like to thank Professor Mikael Pell at the Department of Microbiology, SLU and Lena Rodhe at Swedish Institute of Agricultural and Environmental Engineering, for their kind suggestions and useful comments on experiment design and data analysis. I am particularly grateful for the practical assistances given by Sven Smårs and Evgheni Ermolaev when setting up my experiment. Big thanks to my lovely labmates Marlies Bos and Jenna Senecal for the excellent atmosphere you created at the lab. Last but not the least, thanks for the wonderful time I spent here during the last semester, along with the warming company of all the staff working at the Department of Energy and Technology, SLU.

Xi Yang

Uppsala, September 2013

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1. Introduction

Dewatered sewage sludge produced as end product in the purification process of wastewater at municipal wastewater treatment plants is rich in plant nutrients such as phosphorus (P) and nitrogen (N) (Tenenbaum, 1997) which can be recycled as fertilizers for agricultural purpose. However, sewage sludge also contains organic contaminants and pathogenic organisms which can be toxic and potentially hazardous to health of plants, animals and humans (Tenenbaum, 1997; Kosobucki *et al.*, 2000). It can also result in contamination of the land, especially if the sludge has not been subjected to appropriate treatments prior to land spreading.

In order to sanitize the sewage sludge before recycling the nutrients to land, a storage for one year before being applied to land has been proposed by the Swedish Environmental Protection Agency as minimum requirement. Other measures to reach the sanitation standards are digestion at higher temperatures (thermophilic). However, during the storage the greenhouse gas carbon dioxide (CO_2) is produced by activity of microbes in the sewage sludge and in addition, an earlier study (Flodman, 2002) found that sewage sludge during the storage phase can yield high emissions of methane (CH_4) and nitrous oxide (N_2O). These gases are also considered as great contributor to climate change (IPCC, 2007). N_2O emission during sludge storage can also result in a loss of nutrient in sewage sludge which is primarily aimed to be recycled. Therefore, for better recognizing the potential impact of sludge storage on greenhouse effect, it is important to quantify the amount of greenhouse gas produced during the sludge storage.

Gas emission from stored sludge can be affected by physical, chemical and microbial conditions of the sludge and the ambient environment. Natural evaporation of moisture from the sludge and precipitation can alter the water and oxygen (O_2) content of the stored sludge thus affecting the microbial activities that produces greenhouse gases. During the natural drying process of stored sludge, the upper layer is dried first thus becoming porous and offering an oxic environment for nitrification producing nitrate (NO_3^-) and some N_2O.

One of the hypotheses of this study is that during events of precipitation, NO_3^- produced by nitrification in the upper layers will be percolated with the water through the stored sludge down to the lower anoxic environment and act as a source for denitrification which can produce N_2O. Precipitation contains moisture which can permeate the stored sludge, fill the pore space on its way down and create a larger anoxic environment in the bottom section of the stored sludge which allows methanogens to produce CH_4. Therefore, an increase in emission of both N_2O and CH_4 after irrigating the stored sewage sludge can be expected. Another hypothesis is that drying will make the sludge more aerobic, thus rapidly decreasing the emission of CH_4 which is usually generated by methanogens under anoxic conditions.

The objective of this study was to investigate how drying and rewetting of digested and dewatered sewage sludge affects the emission of greenhouse gases (CO_2, CH_4 and N_2O) during the storage phase at constant room temperature. In order to reach the objective, laboratory experiment was performed and gas emission from two types of digested sludge was investigated.

2. Background

Digested sewage sludge can be managed by various methods including incineration, land spreading and landfill, among which land spreading is the only strategy that can take care of the nutrient. Greenhouse gas can be emitted during these methods of sludge management, but hardly any study could be found focusing on the greenhouse gas emission during the storage phase of dewatered sewage sludge. However, during the anaerobic digestion of sewage sludge, a series of bacteria including hydrolytic bacteria, acetogenic bacteria and methanogens will degrade complex organic matter into smaller molecules such as CO_2 and CH_4 (Bitton, 2011). These bacteria are naturally present bacteria which can also be found in soil systems where many studies on the gas fluxes as well as the impact of drying and rewetting on the dynamics of soil gas fluxes can be found (Kessavalou *et al.*, 1998; Beare *et al.*, 2009; Syamsul *et al.*, 1996; Xu & Luo, 2012; Goldberg & Gebauer, 2009). Therefore, the dynamics of gas fluxes in soil system can be considered as a model for understanding the pathway of gas emission from the activities of bacteria in sewage sludge.

2.1 Pathways of carbon emission

2.1.1 Carbon dioxide

Carbon in soil can be biologically degraded and released into the atmosphere in its gaseous forms CH_4 and CO_2, with the latter one to be the dominant pathway of carbon loss from most belowground environments (Lorenz & Lal, 2010). CO_2 can be produced via cellular respiration where organisms in soil decompose organic matter and generate energy used for maintenance of their life processes. Such respiration occurs in both aerobic and anaerobic environments (Madigan *et al.*, 2012), but differs in the magnitude of CO_2 production. Under oxic condition, microbes utilize O_2 as terminal electron acceptor to respire during which organic carbon can be fully oxidized and degraded into CO_2:

$$(CH_2O)_n + O_2 \rightarrow CO_2 + H_2O \qquad \text{(Equation 1)}$$

When O_2 is absent in the environment, glucose can be converted to CO_2 and ethanol (in heterolactic fermentation, lactic acid is also produced) via fermentation (Equation 2) (Madigan *et al.*, 2012) which indicates an insufficient oxidation of organic carbon and a lower carbon emission as CO_2 compared with aerobic respiration.

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \qquad \text{(Equation 2)}$$

Glucose can also be insufficiently oxidized to CO_2 and CH_4 by hydrolytic bacteria, acetogenic bacteria and methanogens working in close cooperation.

Based on various studies, Davidson (2000) indicated that soil respiration rate was strongly influenced by soil moisture content. A study performed by Doran *et al.* (1990) showed an increase in respiration rate of mixed soil samples as water-filled pore space in soil samples increased from 30% to 61%, with other influencing factors such as temperature and substrate availability being controlled. Further increase in water-filled pore space led to a reduction in respiration rate of the soil samples. Low respiration rate in both wet and dry soil was explained mechanically by wet soil, which had higher water-filled pore space, restricting the diffusion and availability of O_2 in soil (Linn & Doran, 1984) while dry soil resulted in a thin

water film which limited the availability of dissolved organic substrates (Papendick & Camprell, 1981). Additionally, moisture can also limit the respiration rate in the matter by reducing the hydration and activity of enzymes, which can be reactivated once intracellular water potential increases (Stark & Firestone, 1995).

2.1.2 Methane

Compared with CO_2, CH_4 is released in much lower amounts from soil (Lorenz & Lal, 2010), but has a 25-fold greater global warming potential than CO_2 over a 100-year time period (IPCC, 2007). CH_4 in soil is produced under anoxic conditions by methanogens which use some of the carbon from a carbon source (CO_2, acetic acid and other small organic compounds) as terminal electron acceptor and generate CH_4, in a process called methanogenesis (Madigan *et al.*, 2012):

$CO_2 + 4\ H_2 \rightarrow CH_4 + 2H_2O$

or

$CH_3COOH \rightarrow CH_4 + CO_2$ (Equation 3)

Due to the low solubility in water, CH_4 produced via methanogenesis under anoxic conditions will diffuse to oxic environment where it can be either oxidized to CO_2 by methanotrophs contributing to CO_2 emission, or be emitted to the atmosphere (Madigan *et al.*, 2012). Therefore, high CH_4 emission is often found in wetland and water-clogged soil after heavy rainfall or floods where micro pores of soil are mostly water-filled (Lorenz & Lal, 2010).

Methanogens are generally considered as obligate anaerobes (Madigan *et al.*, 2012), which are oxygen sensitive and can only live in the environment devoid of O_2, or even die when exposed to O_2. Long drying period of stored sludge enlarges the gas filled pore space in sludge and facilitates the oxygen diffusion which might lead to a reduction of those anaerobes before imposing rewetting. However, a previous study (Kato *et al.*, 1993) demonstrated that some types of methanogens resided in granular sewage sludge had a high tolerance for O_2 which could be attributed to the O_2 being consumed by facultative microbes. The consumption of O_2 by facultative metabolism formed an anoxic microenvironment where methanogens could be protected.

2.2 Pathways of nitrogen emission

Nitrogen in soil can enter the atmosphere in its gaseous forms, such as ammonia (NH_3), molecular nitrogen (N_2), nitric oxide (NO) and N_2O, a potent greenhouse gas and 297 times more effective in heat trapping compared with CO_2 (IPCC, 2007), via various types of microbial activities. The process of decomposition of organic nitrogen, which produces NH_3, is called ammonification. Some of the volatile NH_3 produced from ammonification in soil can be released to the air if soil is alkaline, otherwise NH_3 will remain in soil as ammonium (NH_4^+) which can either be assimilated by microorganisms (Paredes *et al.*, 2000) or can be oxidized first to nitrite (NO_2^-) and then to NO_3^- (Madigan *et al.*, 2012). N_2, as the most abundant gas in atmosphere of the earth, is produced from soil as the final product of denitrification. NO and N_2O are intermediate products that are mainly produced from nitrification and denitrification (Kim *et al.*, 2012), whereas lower amount of NO was produced from denitrification compared

with that from nitrification (Machefert & Dise, 1999; Skiba *et al*., 1993), while most of the N_2O emission from agricultural lands is produced through denitrification (Cayuela *et al*., 2013).

Nitrification is a biological process carried out by nitrifying microbes, including many species of bacteria and at least one species of archaea (Madigan *et al.*, 2012, Konneke *et al*., 2005). During the process of nitrification, NH_4^+ is first oxidized with the presence of O_2 into NO_2^-, which is then further oxidized into NO_3^- (White, 2005):

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O + \text{energy}$$

$$NO_2^- + 0.5\ O_2 \leftrightarrow NO_3^- + \text{energy} \qquad \text{(Equation 4)}$$

Since nitrification only occurs when the environment is oxic, balance of water/oxygen content in the environment is one of the influencing factors for nitrification. Similar to respiration rate, nitrification rate also increases with availability of water in soil until optimum level of moisture content is reached and the rate will decreased once the water content exceeds that optimum level (Linn & Doran, 1984). Although Barros (1995) indicated that moisture content at field capacity, which is the maximum amount of water that a particular soil can hold after excess water has drained away, was the optimum moisture level for soil microbial activity, 60% of field capacity was suggested by White (2005) as the optimum moisture level specifically for the process of nitrification. However, if soil dries, sufficient nitrifying bacteria can also survive during the period of drying and can be reactivated and again participates in nitrification after soil rewetting (White, 2005).

Besides, pH is also a limiting factor for nitrification. Maximum growth rate of *Nitrosomonas*, one of the bacteria converting NH_4^+ to NO_2^-, is found in soil with pH over 7.6. Optimum pH value for the growth of *Nitrobacter*, which carries out the oxidation of NO_2^- to NO_3^-, is found ranging from 6.6 to 7.6 (White, 2005). Acid released during the nitrification process can result in a decline in pH of the environment and consequently inhibit the growth of the nitrifying microorganisms. However, nitrification process can still be observed when pH of the environment is below 6 (Princic *et al.*, 1998, Tarre & Green, 2004).

Denitrification is a biological stepwise reduction of NO_3^- into NO_2^-, NO, N_2O and eventually molecular nitrogen (N_2) (Knowles, 1982):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad \text{(Equation 5)}$$

These processes usually occur under low oxygen availability (Knowles, 1982). Besides oxygen, pH is also a controlling factor for denitrification. The optimum pH value for denitrification was found to be between 7.0 and 8.0 from a summary of various relevant studies done by Knowles (1982). An experiment performed by (Bremner & Shaw, 1958) showed that denitrification also occurred, but very slowly, in soil with pH between 3.6 and 4.1, and the process became faster when pH was above 5.8. However, pH can also affect the proportion of the products of denitrification. Knowles (1982) demonstrated that the proportion of N_2O produced from denitrification increased as pH value of the soil decreased.

Theoretically, N_2O can also be produced via nitrifier denitrification in soil (Wrage *et al.,* 2001) and abiotic denitrification (Samarkin *et al.*, 2010) in lake systems. However, the most important pathways for N_2O production in soil have long been considered to be nitrification and denitrification (Heincke & Kaupenjohann, 1999; Groffman *et al.*, 2006; Smith *et al.*, 2008), and an experiment has shown that no N_2O was produced from two types of soil via abiotic denitrification process (Cayuela *et al.*, 2013). However, the discovery of the importance of archea for the nitrogen transformations in the soil is fairly recent, and they might have an important role (Gubry-Rangin *et al.*, 2010).

2.3 Previous studies on the effect of drying/rewetting on gas emission from soil

A review covering 222 field and laboratory studies on the effect of soil rewetting on soil gas fluxes was done by Kim *et al.* (2012) showing that a majority of the studies reported an increase in CO_2, N_2O and CH_4 following soil rewetting across various ecosystems. Among all 69 observations on CO_2 and CH_4 emission after soil rewetting, emission from only 8 observations was found to decrease after rewetting. For N_2O, 3 out of 58 observations showed no response or only small-scale increase after rewetting. Two common mechanisms for the general increase in gas fluxes after rewetting was hypothesized by Kim *et al.* (2012); (1) substrate supply increases during drying, and (2) physical mechanisms in relation to moisture change, which have been mentioned in sections 2.1 and 2.2.

3. Methods and materials

3.1 Sewage sludge

In this study, two types of sewage sludge were selected for investigating the gas emission from drying and rewetting processes during storage: (1) mesophilically digested sewage sludge produced by Uppsala Vatten and collected on 18th of March 2013; (2) thermophilically digested sewage sludge produced in Sunne Municipality and collected on 08th of April 2013. Mesophilic digestion was done at average temperature of 37.2 °C with a hydraulic retention time in the reactor of 17 days (Kungsängsverket, 2011). Thermophilic digestion was done at temperature around 49 °C, and the guaranteed minimum retention time in reactor was 3 hours, (Nilsson, personal communication). However, the actual retention time could be much longer than this. At arrival, the sludge was directly put in a fridge at 4 °C until the day experiment started so as to minimize further degradation and microorganism activities in the material.

3.2 Experimental design

In order to imitate the natural top-to-bottom drying process in stored sludge, the sludge in this experiment was stored in cylindrical tubes. In addition, to ensure that ambient environment of stored sludge remained oxic during the whole storage period and to enable gas sampling, each tube was put in an individual jar as the surrounding closed system within which sludge was stored and emitted gas was retained.

3.2.1 Pre-experiment on choosing proper desiccant

To enable drying of sludge stored in a closed space, the relative humidity of the air in the closed space has to be decreased. For this purpose, a desiccant was introduced. It is desirable that the desiccant used should not adsorb any of the gases analyzed in the experiment.

In a pre-experiment, adsorption capacity of the gases CO_2, N_2O and CH_4 of three different types of desiccant was examined: silica gel (SiO_2), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and potassium acetate (CH_3COOK). Each desiccant was put in a sealed vial which was then filled with a standard gas mixture consisting of the gases of interest. The concentration of the gases in the vials was determined 2 weeks later. Negative CO_2 adsorption on CH_3COOK in 2 weeks was found to be 71%. For vials filled with $MgCl_2 \cdot 6H_2O$, 14% of N_2O was adsorbed at 0.502 ppm level while 9% increase in N_2O concentration was found at level of 4.92 ppm. Silica gel did not adsorb any N_2O or CH_4 but there was 30% absorption of CO_2. As a result, silica gel was chosen to perform the drying task and grains of silica gel was scattered over the bottom of the jar where the tube of sludge was placed. The possibility that it would adsorb 30% of the CO_2 emission from stored sludge was handled by a correcting calculation procedure.

3.2.2 Pre-experiment on determining time span of drying/rewetting phase

A pre-experiment was performed to determine the appropriate time span of drying and rewetting. Drying phase was decided to end by the time when the moisture in the sludge was almost half compared to the moisture in the ingoing material. Spent silica gel was then replaced with the same amount of new silica gel as it was at the beginning of the experiment. On the next day, which was the first day of rewetting phase, an irrigation which was roughly

equivalent to 75% of moisture loss from stored sludge during the drying phase was applied onto the sludge surface. The pre-experiment showed that it took 10 days for 60 g of mesophilically digested sludge in a 68 mm diameter tube kept in 3.7 L jar to reach to the desired moisture content level after drying, with the help of 60 g of silica gel. However, the diameter of the tube used in the main experiment was narrower than that in the pre-experiment which would eventually prolong the period of drying, therefore, the drying period of the main experiment was 17 days, and the rewetting period was 15 days.

3.2.3 Pre-experiment on testing leakage of jar

A test for air tightness of the 3.7 L jar, which was used in both pre-experiment and experiment, was done prior to the experiment by monitoring the weight of a closed jar at room temperature for 10 days after placing a tube of 120 g of fresh mesophilically digested sludge and 60 g of silica gel inside. No weight loss from the closed system was found.

3.2.4 Treatments

In order to examine how drying and rewetting affects the greenhouse gas emission from stored sludge, treatment with sludge subjected to drying and rewetting (DR) was first introduced. A control treatment (C) was included in which the sludge was exposed to the same temperature and gas conditions as in the drying/rewetting treatment but almost without any drying as no desiccant was added and therefore there was no irrigation either. The water content of the sludge in the control treatment was expected to stay at a constant level. Furthermore, a third treatment (DRR) was included for tracing the nitrification process during the drying period of the sludge from treatment DR. In this treatment, a small portion (ca. 5 g) of sludge sample from the bottom was collected at the end of the drying period for analyses of content of nitrogen in various forms. Removed sludge was then replaced with fresh sludge stored at 4 °C of same weight. The treatment with the sludge replacement also received the same drying and rewetting actions as those in the first treatment mentioned. The three different treatments stated above (1: drying/rewetting, 2: control, 3: drying/rewetting and sludge replacement) were implemented on two types of sludge, mesophilically digested sludge (M) and thermophilically digested sludge (T), giving the whole experiment six treatments in total (Table 1). Each treatment was carried out in triplicates, resulting in a total of 18 units. All units were kept at room temperature (relatively constant, was tested before experiment started) throughout the experiment.

Table 1. Description of treatments applied in experiment.

Treatment ID	Treatments			
	Substrate	Drying and rewetting	Sludge replacement	Repetitions (IDs)
MDR	Mesophilically digested sludge	applied	none	3 (M1,M2,M3)
MDRR	Mesophilically digested sludge	applied	applied	3 (M4,M5,M6)
MC	Mesophilically digested sludge	none	none	3 (M7,M8,M9)
TDR	Thermophilically digested sludge	applied	none	3 (T1,T2,T3)
TDRR	Thermophilically digested sludge	applied	applied	3 (T4,T5,T6)
TC	Thermophilically digested sludge	none	none	3 (T7,T8,T9)

3.3 Experiment set-up

60 g of sludge sample, which is roughly equivalent to 100 mL in volume, was collected from the well-mixed sludge and transferred to a 120 mL flat based HD-PE cylindrical tube (42 mm diameter, 114 mm height). Gentle vibration and tapping was applied to the tube to compact the sludge for reducing the pore space in the bottom thus forming a non-aerated environment. The tube was then placed in a 3.7 L HD-PE jar with a screw lid in which a hole had been drilled and equipped with a rubber plug to enable gas sampling (Figure 1). For treatments where drying was applied, 90 g of silica gel with a color moisture indicator and a grain size of 1-4 mm was scattered over the bottom of the jar before placing the tube with sludge inside.

Figure 1. HD-PE jar with lid attached. Personal photograph by Xi Yang. 18 Sept 2013.

The used silica gel was replaced with 90 g of fresh silica gel on the last day of the drying period to prevent from being over saturated after rewetting. Rewetting was done by drip irrigating 15 g of deionized water from surface of sludge using a 30 mL syringe. The purpose of drip watering was to make sure that all water irrigated was first soaked up by the top of stored sludge until saturate and then infiltrated through the sludge cylinder down to its bottom.

The 18 units were divided into three blocks and located on a five-shelf bookcase according to randomized block design (Figure 2). Unit M2, M4, M9, T1, T5 and T7 belonged to block 1, unit M3, M6, M8, T3, T6, T8 belonged to block 2 while units M1, M5, M7, T2, T4 and T9 belonged to block 3.

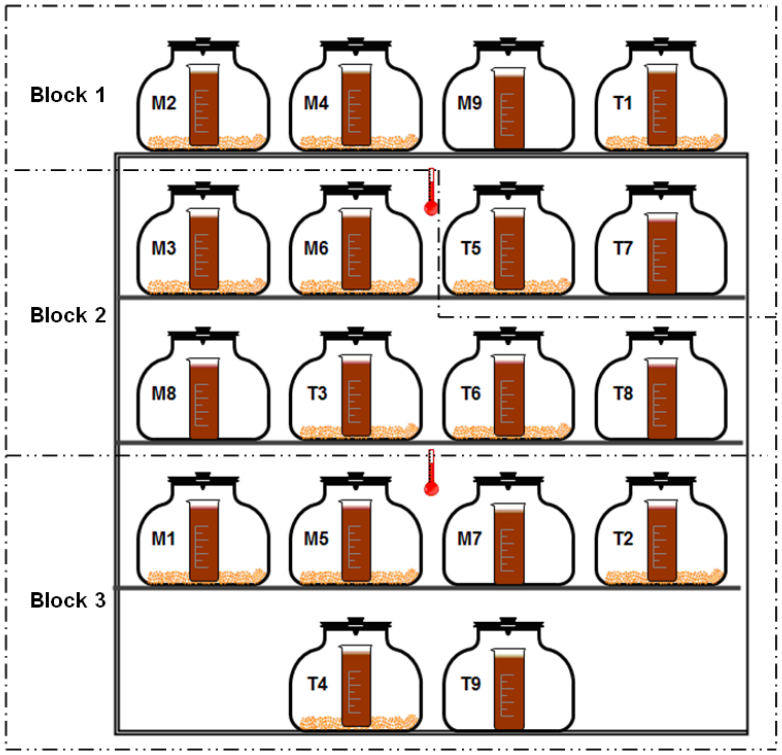

Figure 2. Locations of units and temperature sensors on shelf. Temperature sensor E10 was located in block 3 between unit M5 and M7, sensor 428 was placed between unit M6 and T5.

Two digital thermometer sensors named E10 and 428 were installed at second and fourth shelf, respectively (Figure 2). Temperature during the experiment was recorded continuously by software LogTemp, with 15 minutes sampling interval. Experiment schedule including date, samplings and measurements is shown in Table 2.

Table 2. Schedule of the experiment. Rewetting was performed on day 17, after the gas sampling and weight measurement on the same day

2013-04-15	2013-04-16	2013-04-17	2013-04-18	2013-04-19	2013-04-20	2013-04-21
Day0	Day1	Day2	Day3	Day4	Day5	Day6
W,N,A	E,C	E,C	E	E,C	E	E
2013-04-22	2013-04-23	2013-04-24	2013-04-25	2013-04-26	2013-04-27	2013-04-28
Day7	Day8	Day9	Day10	Day11	Day12	Day13
E,C	E,W	E,C		E,C		
2013-04-29	2013-04-30	2013-05-01	2013-05-02	2013-05-03	2013-05-04	2013-05-05
Day14	Day15	Day16	Day17	Day18	Day19	Day20
E,C,W		E,C,N½,W,R	I,E,W	E,C	E	E,C
2013-05-06	2013-05-07	2013-05-08	2013-05-09	2013-05-10	2013-05-11	2013-05-12
Day21	Day22	Day23	Day24	Day25	Day26	Day27
E	E	E,C	E,W	E,C		E,C
2013-05-13	2013-05-14	2013-05-15	2013-05-16	2013-05-17		
Day28	Day 29	Day 30	Day 31	Day 32		
		E,C		E,C,N,W		

W: Weighing of materials; N: Sludge sampling for chemical analysis and dry matter (TS)/ volatile solids (VS) analysis; A: Sludge sampling for Agrilab analyses; E: Gas sampling for drying/rewetting (experiment) treatments; C: Gas sampling for control treatments; N½: Mid-term sludge sampling for chemical analysis; R: Silica gel replacement; I: Sludge irrigation

3.4 Materials weighing

Seven weighing measurements were done during the experiment (see Table 2). Weights were recorded for: tube with stored sludge, jar with silica gel (excluding the lid) and the whole closed unit after each gas sampling. Measurements were done by a digital weighing scale with 10 mg of readability. Gained weight of silica gel indicated how much moisture had been lost from stored sludge while weight loss from stored sludge represented the loss of both moisture and degraded solids. From this, estimated dry matter of sludge on each measurement day could be calculated.

3.5 Gas sampling and gas chromatography

Gas sampling was performed at 24 occasions whereof 15 occasions included sampling from the units in the control treatments (see Table 2). On each gas sampling occasion, two 50 mL gas samples per jar were collected by an airtight 60 mL syringe through the rubber plug on the jar lid and separately filled in two capped and evacuated PerkinElmer 22 mL crimp top borosilicate glass vials. Gas was pumped through the syringe three times before collecting each gas sample to ensure a uniform sample. To maintain the pressure of each gas sample at the same level as atmosphere pressure, 50 mL of nitrogen gas was added prior to each individual gas sampling. After finishing all gas sampling on each occasion, all the jars were ventilated by removing the lids for around 5 minutes during which ventilation by fanning five times above all units was performed. Six air samples determining the background concentration was collected when the jars were open. Time of start and end of each ventilation as well as time when each gas sample was collected was recorded for gas emission analyses.

Two gas samples taken from the same unit on the sampling day were separately analyzed by two Clarus 500 Gas Chromatographs for different components. One machine was equipped with a flame ionization detector (FID) and an electron capture detector (ECD) for analyzing CH_4 and CO_2, respectively. The other machine used for detecting N_2O and CH_4 was equipped with a thermal conductivity detector (TCD) and a FID detector. Concentration of gas in each sample was calculated based on a calibration curve from the responses of gas standards at known concentration.

3.6 Sludge sampling and chemical analyses

Chemical analyses of sludge in this experiment included pH analysis, ammonium nitrogen (NH_4-N) analysis, nitrate nitrogen (NO_3-N) analysis and total nitrogen (tot-N) analysis. For pH analysis the sludge samples were diluted 1:4 with deionized water. pH in the sludge suspension was measured at room temperature by Meterlab® PHM210 Standard pH Meter. Calibration of pH meter was done each measurement day prior to the measurements. Concentration of different forms of nitrogen in sludge sample was measured by Spectroquant® NOVA 60A photometer, using methods provided by Spectroquant® (NH_4-N: 114559, NO_3-N: 114764 and tot-N: 114763). Based on the chemical parameter analyzed, sludge sample (1:4 diluted) was at some occasions further diluted with deionized water to meet the measuring range of the method.

During the experiment, three sludge samplings were done for chemical analyses (Table 2). The first sludge sampling was done on the day when experiment started. Three 10 g sludge samples per substrate were taken from the sludge and stored in fridge at 4 °C until being analyzed four days later. On the last day of drying phase, one 5 g sludge sample was scooped out from the bottom of each sludge cylinder in the units belonging to treatments MDRR and TDRR. Last sludge sampling was done at the end of the experiment following the gas sampling that day. One 10 g of sludge sample was taken from the top of each tube of stored sludge in all six treatments and another 10 g of sample was taken from the bottom. Each sample collected from the last sludge sampling was spilt up into two 5 g of sub-samples from which one was sent to chemical analyses and the other was measured for TS and VS.

On the first day of the experiment, 500 g of sludge sample taken from each substrate were transferred and kept in a -27 °C freezer until being analyzed for chemical components by Agrilab AB on 16th of May, 2013. Parameters included in Agrilab analyses were: TS, total nitrogen (tot-N), organic N, NH_4-N, total carbon (tot-C), ratio of tot-C to tot-N, total P, total potassium (K), total magnesium (Mg), total calcium (Ca), total sodium (Na) and total sulfur (S). Methods used for the analyses of the parameters are listed in Table 3.

Table 3. Parameters analyzed by Agrilab with the methods used for measurements. Method for analyzing organic nitrogen is unknown.

Parameters	Methods and reference numbers	Accuracy of measurement
pH	SS-ISO 10 390	± 0.2
Total P, K, Mg, Ca, Na, S	SS 28311	± 15 %
Tot-C	SS-ISO 10694	± 15 %
Tot-N	SS-ISO 13878	± 15 %
NH_4-N	Extraction with 2 M KCl and measured by Flow-injection Analysis	± 15 %
TS	KLK 1965:1	<1 %

3.7 Dry matter and volatile solids analysis

TS and VS in sludge were measured according to European Standard: WI CSS99022 and WI CSS99023 developed by Horizontal project (HORIZONTAL, 2007). At the start of the experiment, three sludge samples of 20 g were taken from each substrate and analyzed for TS and VS. Also, TS and VS analyses of 5 g of sub-samples originating from the final sludge sampling for chemical analyses mentioned above were performed on the day the samples were divided for different analytical purposes. Evaporating dishes used in the TS and VS analyses were disposable and made of aluminum. The initial drying time of TS analysis was 14 hours while initial high temperature ignition process of VS analysis was set as 3 hours. Temperature used for drying process in TS analysis was 105 °C and ignition temperature was set to be 550 °C according to the methods used.

4. Result

4.1 Temperature

During the experiment, the temperature sensors E10 and 428 reported 2929 and 3039 measurements respectively. Average of temperature of ambient air measured by sensor E10 was 22.03 °C with a standard deviation (SD) of 0.34 °C, whereas the mean value of ambient air temperature provided by sensor 428 was 22.07 °C with a SD of 0.33 °C. Minimum and maximum temperatures measured by sensor E10 and sensor 428 were 20.81 and 20.94 °C, and 23.63 and 23.50 °C. Average temperature during the whole experiment was 22.05 °C.

4.2 Changes in moisture, dry matter and volatile solids of sludge

4.2.1 Dry matter and volatile solids analysis

Table 4. TS and VS content of both substrates sampled at the start of the experiment. Table shows, for each substrate and parameter, the mean for three repetitions (n = 3). Standard error (SE) of the mean was given.

	TS (%)		VS (% of TS)	
	Mean	SE	Mean	SE
Mesophilically digested sludge	19.90	0.03	68.12	0.06
Thermophilically digested sludge	27.04	0.11	55.21	0.05

When determining TS content of sludge sampled on day 0, two further drying processes (1 h per each process) were applied after the initial 14 hours drying period, until constant mass of residue on the dish was reached. Weight of residue on ignition did not reach a constant level until two extra ignition processes (1 h per each process) were added after the first 3-hour ignition. Overall, the mesophilically digested sludge had a higher initial content of moisture and volatile solids compared with the thermophilically digested sludge (Table 4).

Table 5. TS and VS of sludge sampled from top and bottom layers at the end of experiment (n = 3)

Sampling location	Treatment	TS (%)		VS (% of TS)	
		Mean	SE	Mean	SE
Top	MDR	51.87	0.47	34.61	0.08
	MDRR	50.00	0.83	34.99	0.04
	MC	21.36	0.16	35.92	0.27
	TDR	56.71	1.67	47.75	0.02
	TDRR	58.03	0.91	47.31	0.29
	TC	28.29	0.32	47.48	0.11
Bottom	MDR	18.84	0.02	35.54	0.04
	MDRR	18.48	0.11	35.10	0.05
	MC	18.31	0.08	35.71	0.08
	TDR	32.32	0.67	48.12	0.38
	TDRR	31.40	0.40	48.76	0.12
	TC	26.66	0.08	47.32	0.31

Table 5 shows the result from TS and VS analyses of stored sludge on the last day of the experiment. Only one extra 1-hour of drying and 1-hour of ignition were needed in addition to the initial drying and ignition processes for obtaining a constant residue mass. Dry matter of samples taken from the top layer of the sludge cylinder from treatments MDR, MDRR, TDR and TDRR more than doubled compared with their initial TS content (Table 4 and Table 5). A greater reduction in volatile content of mesophilically digested sludge during the experiment was found compared with that of thermophilically digest sludge, regardless of treatments and sampling location.

4.2.2 Moisture loss and estimated dry matter content

According to the dry matter content of each substrate measured in the beginning of the experiment, there was 48.06 g and 43.77 g of moisture in 60 g of mesophilically and thermophilically digested sludge respectively on the day the experiment started. During the whole drying phase, about 39% of moisture in mesophilically digested sludge from treatment MDR and MDRR (around 19 g) had been lost with the help of desiccant while sludge from treatment TDR and TDRR had lost 46% of its moisture (around 20 g).

During the 15-day drying after the rewetting, approximately 37% (16-17 g) of moisture was evaporated from sludge in treatments MDR and MDRR while moisture loss from sludge during this period in treatments TDR and TDRR was 46-47% (18-18.5 g). Estimated dry matter of sludge from control treatment MC and TC shown below was calculated based on assumption that moisture loss from sludge in both control treatments was equal to its total loss in mass. Therefore, only 5% and 6% of moisture loss of the stored sludge from treatment MC and TC was measured during the whole 32-day storage phase. Estimated dry matter content of sludge on each weight measuring day is listed in Table 6.

Table 6. Development of estimated dry matter (%) of stored sludge during both initial drying phase and the drying phase after rewetting (n = 3)

Treatment	Parameter	TS (%)							
					Day 17				
		Day 8	Day 14	Day 16	Before irrigation	After irrigation	Day 24	Day 25	Day 32
MDR	Mean	23.68	26.65	26.88	27.92	20.35	25.12	nm	30.47
	SE	0.14	0.19	0.24	0.21	0.13	0.26		0.32
MDRR	Mean	23.20	26.07	26.53	27.16	19.82	24.05	nm	29.03
	SE	0.41	0.51	0.52	0.56	0.40	0.54		0.69
MC	Mean	nm	20.31	nm	nm	nm	nm	20.63	20.78
	SE		0.01					0.02	0.02
TDR	Mean	32.97	37.54	38.46	39.78	28.86	36.54	nm	44.16
	SE	0.14	0.15	0.15	0.11	0.07	0.23		0.46
TDRR	Mean	33.40	37.95	38.98	40.19	29.08	36.92	nm	44.80
	SE	0.14	0.13	0.12	0.09	0.05	0.20		0.21
TC	Mean	nm	27.54	nm	nm	nm	nm	28.02	28.28
	SE		0.03					0.06	0.07

nm = not measured

4.3 Gas emission during the experiment

4.3.1 Emissions of carbon dioxide

4.3.1.1 Daily emission of carbon dioxide

Daily emitted CO_2 from stored sludge of all six treatments is shown in Figure 3. Unit of the CO_2 emission was µg CO_2-C g^{-1} tot-C d^{-1}, in which tot-C stands for the substrate initial total carbon content measured by Agrilab. CO_2 daily emission in all six treatments showed an overall decline during the whole experiment. CO_2 emitted from mesophilically digested sludge remained higher than that from thermophilically digested sludge during the whole experiment. Notice that one vial of gas sample taken on day 5 from unit T2 (belonged to treatment TDR) was broken during transportation. Therefore, CO_2 concentration of the gas in the vial could not be measured.

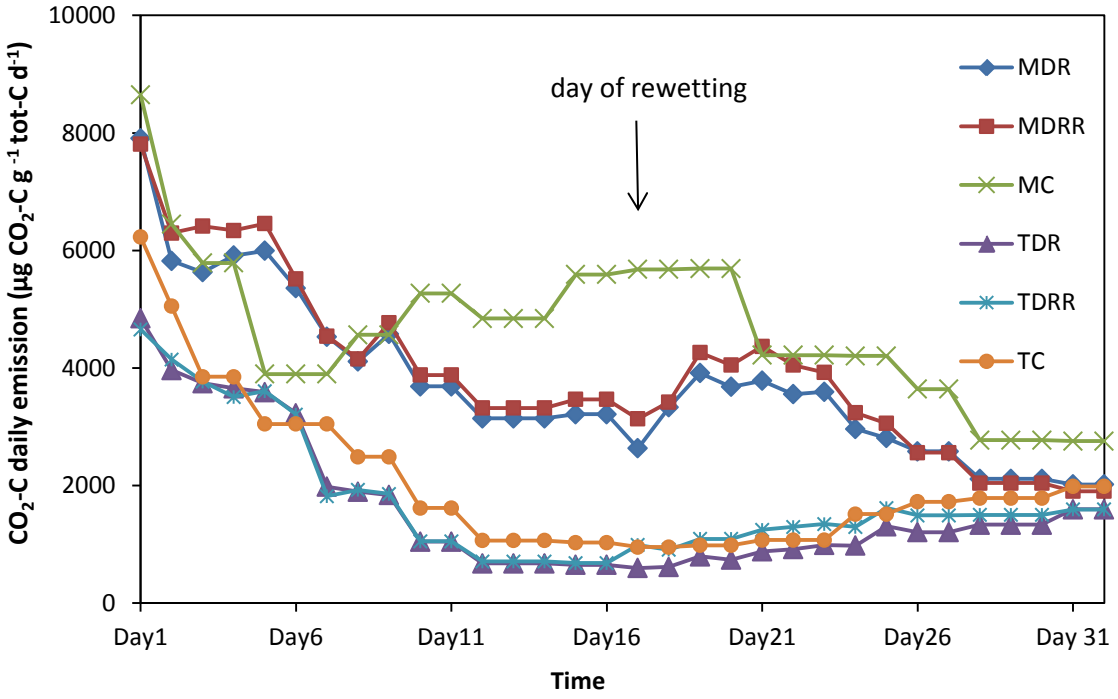

Figure 3. Effect of different treatments on daily emitted CO_2-C during the whole storage phase. Irrigation was done on day 17. Graph shows, for each treatment, the mean for three repetitions (n = 3). Note that for treatment TDR, there was one occasion (day 5) that n=2; for the rest of the occasions in treatment TDR, n=3.

During the first week of drying phase, CO_2 emission from treatment MC decreased more quickly (by over 50%, from 8651 to 3898 µg CO_2-C g^{-1} tot-C d^{-1}) than that from MDR and MDRR (around 40%). However, during the following 10 days before rewetting, CO_2 emission from MC treatment started to increase and leveled off at 5500~5600 µg CO_2-C g^{-1} tot-C d^{-1} while MDR and MDRR treatments maintained the trend of reduction until rewetting was applied. The difference in emitted CO_2 between treated groups and control group for stored thermophilically digested sludge was less obvious, but the CO_2 emission from control stayed slightly higher than that from treatments TDR and TDRR during the drying phase.

As expected, a sudden upward trend in CO_2 daily emission after irrigation was found in treatments applied with rewetting. However, this increase was much more moderate in treatments TDR and TDRR than in treatments MDR and MDRR. After irrigation, daily CO_2 emission from treatment MDR and MDRR reached their peak around 2-4 days after irrigation, followed by a gradual decline during the rest of the experiment after rewetting. CO_2 produced from treatment MC, which was not rewetted, on each day also dropped from day 21 till the end of the experiment. In contrast, daily emitted CO_2 from thermophilically digested sludge in all three treatments after the rewetting showed a small-scale increase compared with the daily emission before rewetting. Even for the control treatment TC, which was not rewetted, the CO_2 daily emission started to climb up from day 21. Additionally, sludge in treatments MDRR and TDRR contributed to a higher daily CO_2 production during the whole experiment after the rewetting compared with treatment MDR and TDR, respectively.

According to the results from the trial experiment on gas adsorption capacity on silica gel, 30% of CO_2 in the vials have probably been adsorbed on silica gel during 2 weeks. It was assumed that CO_2 was gradually adsorbed by the new silica gel during the first three days at the pace of 10% adsorption per day, which resulted in 30% adsorption in total without any further CO_2 adsorption until the day silica gel was disposed. Therefore, during the first 3 days of drying period and rewetting period, actual CO_2 production from sludge stored with desiccant silica gel could be up to 10% higher than the value shown in Figure 3.

4.3.1.2 Accumulated emissions of carbon dioxide

Accumulated production of gas during the whole experiment was obtained by adding up the daily production on each day over the whole experiment. However, in order to calculate the actual accumulated emission of CO_2, the additional portion of CO_2 which was assumedly adsorbed by silica gel was added to the sum of the calculated daily CO_2 production. CO_2 adsorbed by silica gel was determined with the formula:

$$\text{Adsorbed } CO_2 = \frac{\text{total emitted } CO_2 \text{ on the day before disposal of silica gel}}{0.7} \times 0.3 \qquad \text{(Equation 6)}$$

Note that silica gel was disposed twice. Equations 6 was established based on the assumption that, firstly, 30% of ambient CO_2 could be adsorbed by silica gel when equilibrium was reached and, secondly, the CO_2 captured in the silica gel was not released to the air during the short time of ventilation after each gas sampling.

In general, mesophilically digested sludge emitted more CO_2 than thermophilically digested sludge during the whole storage phase, ranging from 123366 to 148681 µg CO_2-C g^{-1} tot-C from the mesophilically digested treatments compared with 53089 to 64501 µg CO_2-C g^{-1} tot-C produced from the thermophilically digested treatments (Figure 4). Higher CO_2 production was found in control treatments than treatments with drying and rewetting, however the SE of both control treatments were larger compared to the other treatments. Total emitted CO_2 from treatments MDRR and TDRR were only slightly higher than those in treatments MDR and TDRR, respectively.

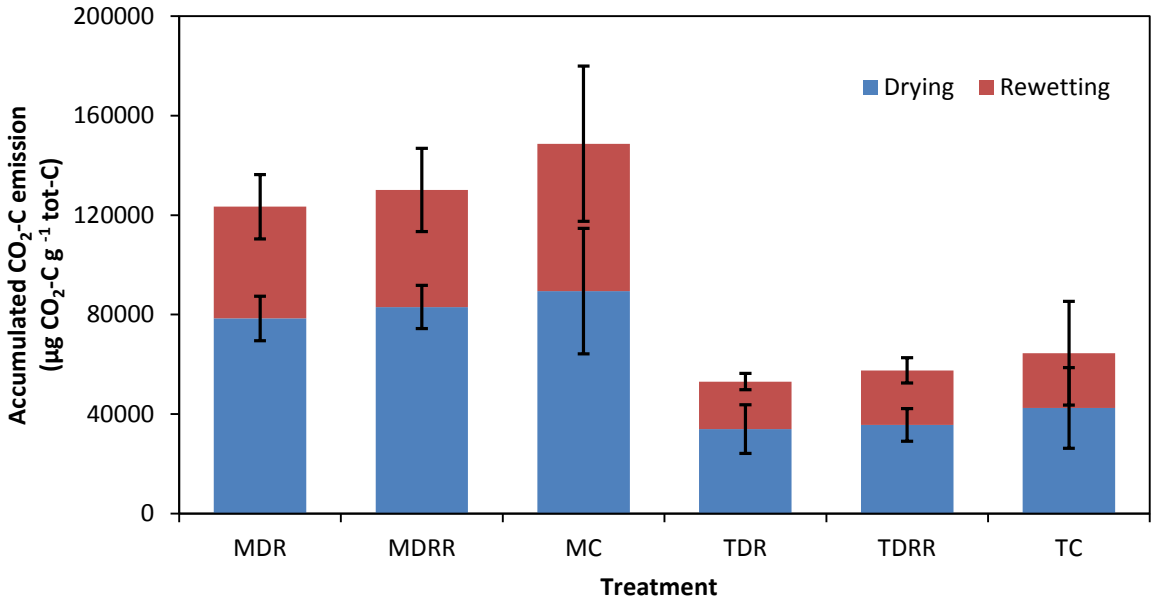

Figure 4. Accumulated CO_2-C emission from all treatments during drying phase and 15 days of period after rewetting. Error bars represent 95% confidence interval. For each treatment, n = 3. Note that the missing data of daily CO_2 production from unit T2 on day 5 might result in a wider confidence interval for the mean accumulated CO_2 production for treatment TDR.

4.3.2 Emissions of methane

4.3.2.1 Daily emissions of methane

The trend of daily CH_4 emission from stored sludge in all treatments is shown in Figure 5. Unit of the CH_4 emission was µg CH_4-C g^{-1} tot-C d^{-1}, in which tot-C stands for the substrate initial total carbon content measured by Agrilab.

Compared with the daily emission of the two other gases, the amount of daily emitted CH_4 was considerably lower, especially for the CH_4 produced from thermophilically digested sludge. Highest CH_4 production rate during the whole storage phase appeared on the first day of the experiment. First day production of CH_4 from sludge in treatment MDR was the highest (234 µg CH_4-C g^{-1} tot-C) among all treatments, followed by MC and MDRR being 154 and 146 µg CH_4-C g^{-1} tot-C, respectively. CH_4 emitted from stored thermophilically digested sludge on the first day ranged from 13-21 µg CH_4-C g^{-1} tot-C. After day 7, CH_4 daily production from all six treatments reduced to less than 0.1 µg CH_4-C g^{-1} tot-C d^{-1}. Peaks in daily emission of CH_4 following irrigation was found in all treatments except MC and TC, and among those peaks, the highest was shown in MDRR (50 µg CH_4-C g^{-1} tot-C d^{-1}) on third day after sludge rewetting while treatments MDR, TDR and TDRR peaked at 0.02-0.03 µg CH_4-C g^{-1} tot-C d^{-1} on the same day (since daily emitted CH_4 before irrigation was negative, 0.02~0.03 µg CH_4-C g^{-1} tot-C d^{-1} was still considered as a peak though the value was negligibly low).

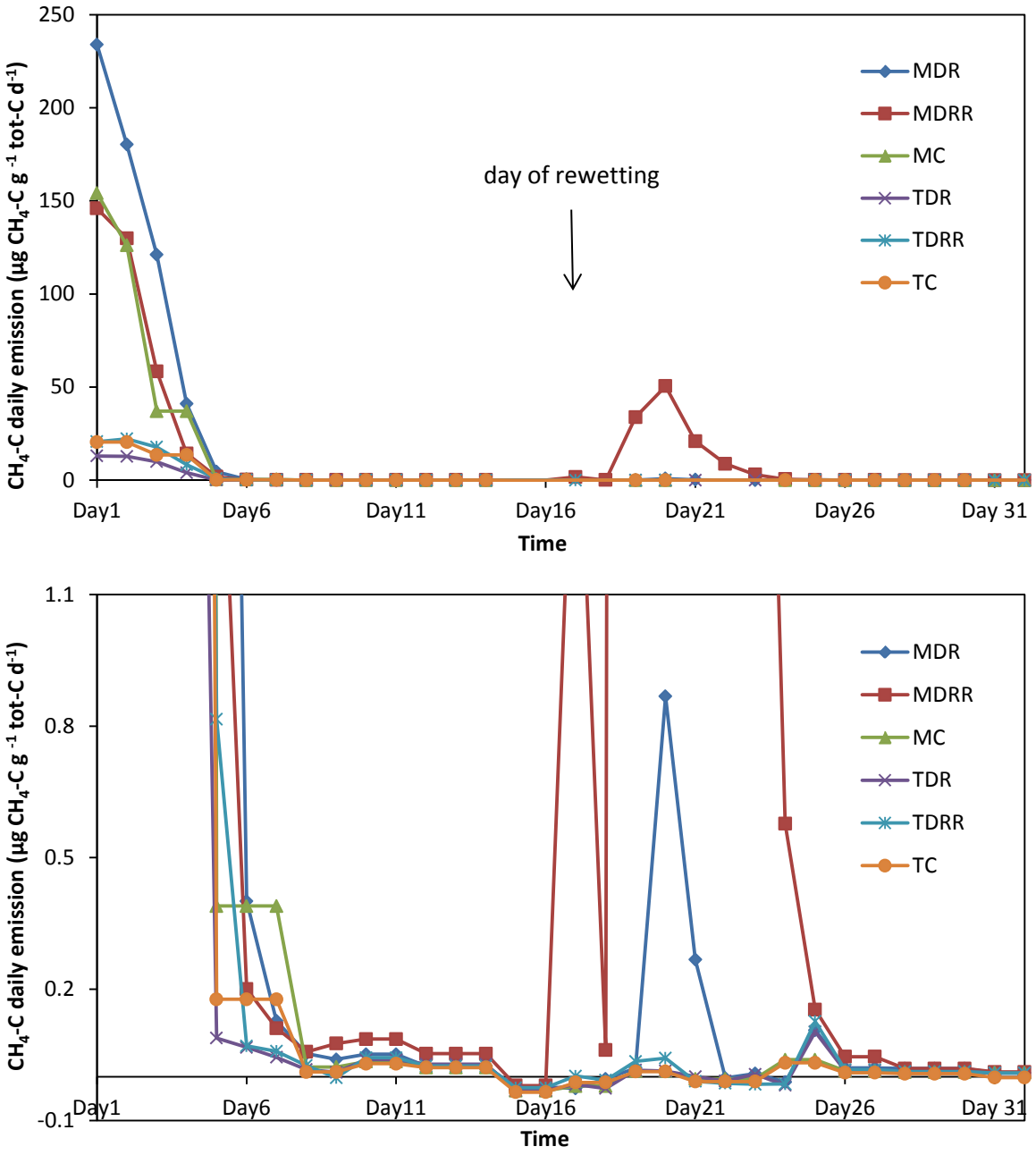

Figure 5. Effect of different treatments on daily emitted CH_4-C during the whole storage phase. Irrigation was done on day 17. For each treatment, n = 6. Note that for treatment TDR, there was one occasion (day 5) that n=5; for the rest of the occasions in treatment TDR, n=6. Since the CH_4 emissions during most of the experiment were lower than 1 µg CH_4-C g $^{-1}$ tot-C d^{-1}, an individual graph with y-axis showing emissions between -0.1 and 1.1 µg CH_4-C g $^{-1}$ tot-C d^{-1} was added.

Since both gas chromatographs had a FID installed for detecting CH_4, the analyses of CH_4 concentration of gas samples taken from each jar was twice as many as for the other gases; daily emitted CH_4 from each treatment was determined by six gas samples instead of three. However, one vial of gas sample taken on day 5 from unit T2 (belonged to treatment TDR) was broken during transportation. Therefore, CH_4 concentration in unit T2 on day 5 was measured only by one GC machine instead of two. Also note that since CH_4 concentration of air samples included in GC analysis on 15th of May (sampled from day 20 to day 25) were

reported with false-low values, calculated CH_4 concentration of both air and gas samples from day 20 to day 25 were determined by a calibration curve which was created by joining the average intercept of calibration curves from the rest of the GC runs (indicating value of detected area at 0 ppm of CH_4) and the average of detected area of air samples in the same run (indicating value of detected area at ambient concentration). False-low values of detected area of CH_4 provided by GC on 15th of May could be the consequence of a short-time power failure that happened before the GC analysis on that day, or else some other unknown reasons.

4.3.2.2 Accumulated emissions of methane

Total emitted CH_4 during the whole experiment (Figure 6) was much lower than the production of the other two gases. The highest amount of total CH_4 was 582 µg CH_4-C g^{-1} tot-C and was emitted by treatment MDR. The substrate was a strong influencing factor in terms of CH_4 production from the sludge. The joint average of total emitted CH_4 from treatments MDR, MDRR and MC was almost seven times higher than the joint average of CH_4 produced from treatment TDR, TDRR and TC. The drying process did not affect the CH_4 emission much. The rewetting hardly contributed to any CH_4 production during the period after the rewetting except for treatment MDRR. A difference in accumulated CH_4 emission during drying phase was found between MDR and MDRR and also between TDR and TDRR.

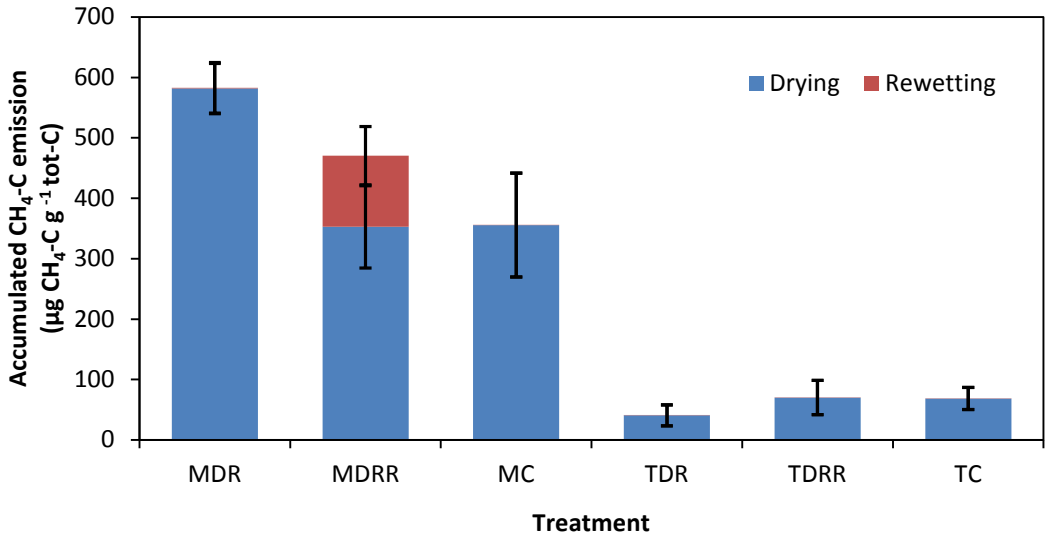

Figure 6. Accumulated CH_4-C emission from all treatments during drying phase and 15 days of period after rewetting. Error bars represent 95% confidence interval. For each treatment, n = 6. Note that the missing data of daily CH_4 production from unit T2 on day 5 might result in a wider confidence interval for the mean accumulated CH_4 production for treatment TDR.

4.3.3 Emissions of nitrous oxide

4.3.3.1 Daily emission of nitrous oxide

N_2O emissions was expressed as µg N_2O-N g $^{-1}$ tot-N d^{-1}, in which tot-N stands for the substrate initial total nitrogen content measured by Agrilab. Unlike daily emitted CO_2, which was high at the start of the experiment followed by a general decline during the whole storage phase, there was hardly any N_2O emitted from any of the six treatments (around 8~9 µg N_2O-N g^{-1} tot-N d^{-1}) during the first two days of the drying period (Figure 7). A boom in N_2O daily production was observed from the third day and the emissions from all treatments continue to

increase rapidly until they peaked on the 6th day of the experiment. The emission from MC treatment was the lowest (6546 µg N_2O-N g^{-1} tot-N d^{-1}) and emission from the treatment TDR was the highest (10447 µg N_2O-N g^{-1} tot-N d^{-1}) among all treatments. Daily emitted N_2O from treatment MC and TC steadily reduced after the peak till the end of the experiment, whereas the N_2O emission rate from the other four treatments bounced up to a second peak on the day after rewetting. Compared with the great gap of CO_2 production rate between mesophilically digested sludge and thermophilically digested sludge, no noticeable difference was found in daily emitted N_2O between the two substrates (Figure 7).

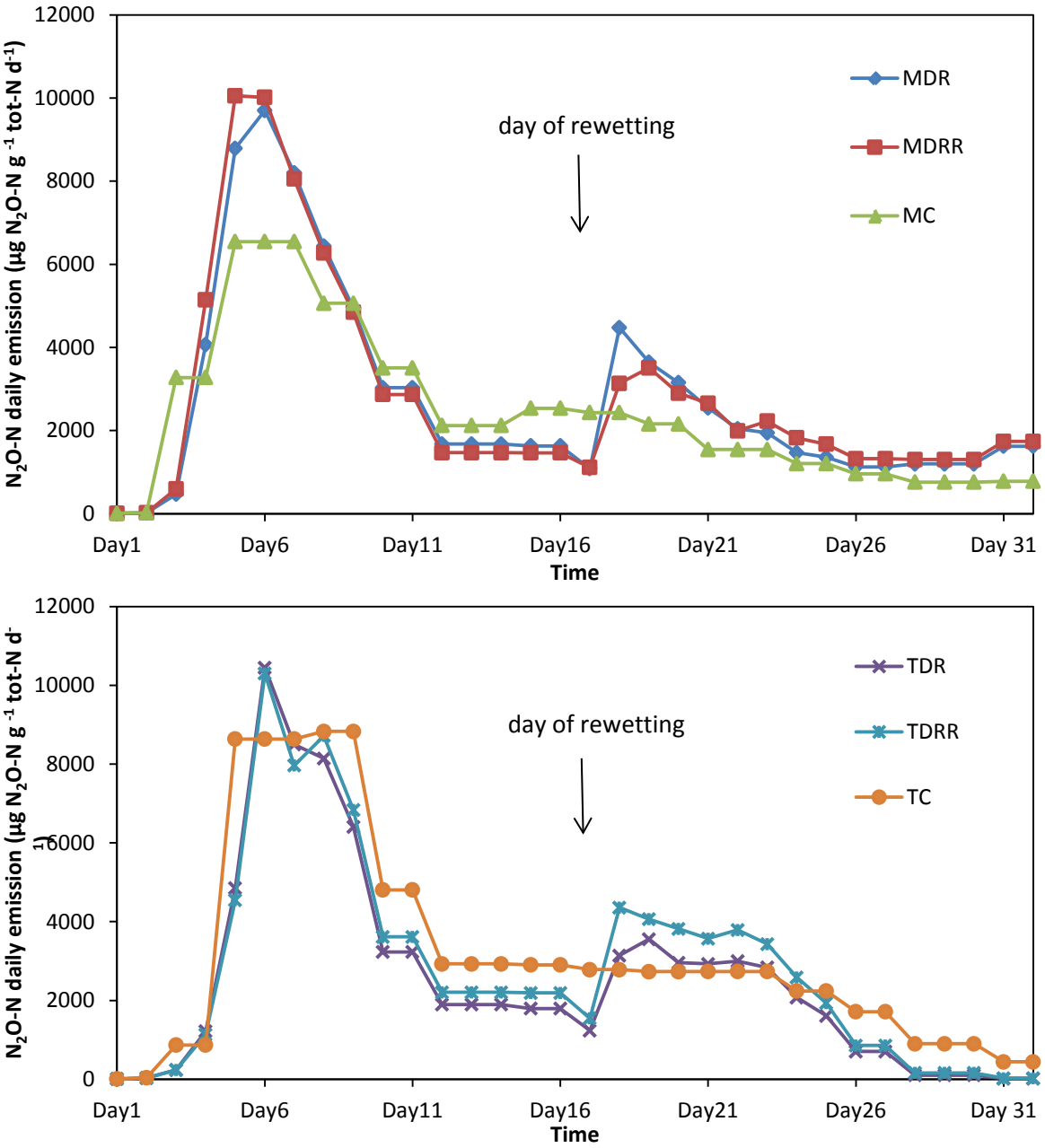

Figure 7. Effect of different treatments on daily emitted N_2O-N during the whole storage phase. Note that since few gas samples were not fully detected by GC, emission from treatment MC and TC on day 5-7 could be even higher than what is shown in the graph. Irrigation was done on day 17. For each treatment, n = 3. Since all the treatments followed the same pattern, the data is split into two graphs to be easier to read.

4.3.3.2 Accumulated emissions of nitrous oxide

The different treatments did not show any great difference in accumulated N_2O emission during the whole experiment (Figure 8). However, minor variance was found between treatments with the same substrate. Accumulated N_2O emission from control treatment MC during the whole experiment (76760 µg N_2O-N g^{-1} tot-N) was lower than those from treatment MDR (87760 µg N_2O-N g^{-1} tot-N) and MDRR (89097 µg N_2O-N g^{-1} tot-N) while total produced N_2O from control treatment TC (100292 µg N_2O-N g^{-1} tot-N) was higher than those from TDR (80672 µg N_2O-N g^{-1} tot-N) and TDRR (89357 µg N_2O-N g^{-1} tot-N).

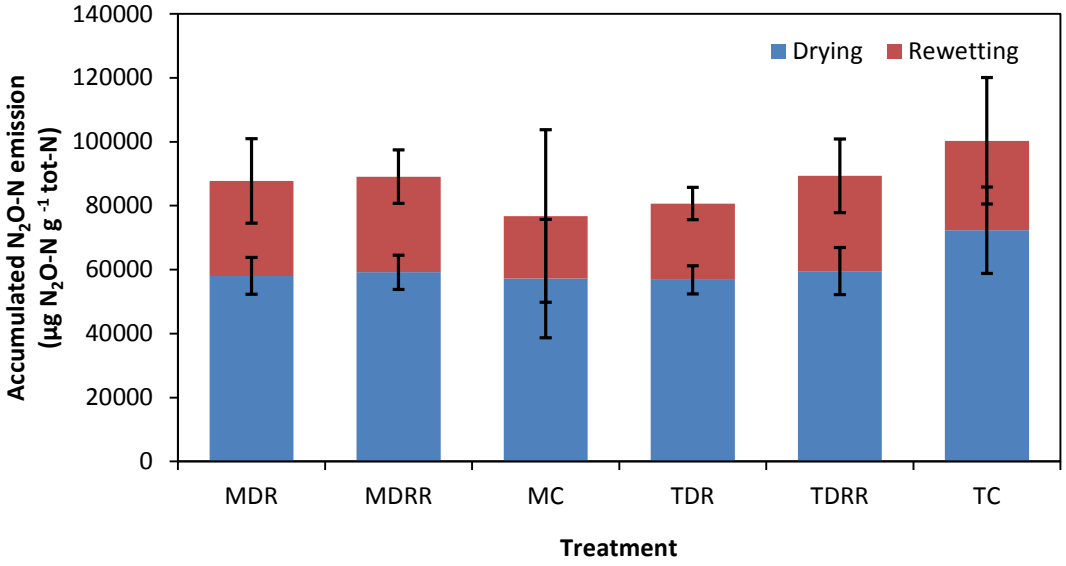

Figure 8. Accumulated N_2O-N emission from all treatments during drying phase and 15 days of period after rewetting. Error bars represent 95% confidence interval. For each treatment, n = 3. Note that actual emission from treatment MC and TC during drying process could be slightly higher than that shown in the figure.

4.4 Chemical parameters

Concentrations of chemical parameters tot-N, NH_4-N and NO_3-N were expressed as gram of chemical per kilogram of dry matter (g kg^{-1} TS) (Table 7). There was no TS analysis done for the second sludge sampling due to insufficient amounts of sludge available, therefore, the assumption was made for calculating chemical concentration in the sludge sampled one day before irrigation (day 16) that the dry matter of the sludge sampled on that day was equal to the initial dry matter content of each substrate (19.90% for mesophilically digested sludge and 27.04% for thermophilically digested sludge). Note that chemical analyses for the third sludge sampling were done 7 days after sludge samples were taken due to unavailability of the spectrophotometer at lab. Sludge samples were kept in fridge at 4 °C until being analyzed. For data with standard error, see Appendix 1.

Table 7. Nitrogen concentrations and pH of sludge samples on day 0, 16 and 32 of the storage experiment. Note that no pH measurement was done for sludge sampled on day 16.

Sampling date, location and treatment		Mesophilically digested sludge				Thermophilically digested sludge			
		NH_4-N	NO_3-N	Tot-N	pH	NH_4-N	NO_3-N	Tot-N	pH
		g kg^{-1} TS	g kg^{-1} TS	g kg^{-1} TS		g kg^{-1} TS	g kg^{-1} TS	g kg^{-1} TS	
Day 0		6.8	0.1	90	7.46	6.7	0.0 [1)]	36	7.54
Day 16, bottom	DRR	3.5	2.4	52	--	1.6	0.4	49	--
Day 32, top	DR	2.7	2.3	23	6.42	2.2	0.6	20	6.41
	DRR	2.8	2.6	17	6.32	2.1	0.6	9	6.42
	C	5.5	5.2	52	6.12	1.3	1.6	40	6.10
Day 32, bottom	DR	0.1	4.9	47	4.84	0.0 [2)]	0.1	27	6.24
	DRR	0.1	4.0	55	5.59	0.0 [3)]	0.0 [4)]	30	6.36
	C	4.0	6.6	59	5.49	0.2	1.4	28	5.95

1) 0.045 g kg^{-1} TS; 2) 0.011 g kg^{-1} TS; 3) 0.006 g kg^{-1} TS; 4) 0.015 g kg^{-1} TS

Results of chemical analyses of sludge sampled specifically for Agrilab AB can be seen in Table 8. Units of the parameters of chemical concentration were given as kg ton^{-1}, which stands for kg of chemical per ton of fresh sludge. The most noticeable difference between the substrates is the carbon/nitrogen (C/N) ratio, where mesophilically digested sludge was richer in tot-N and organic N than the thermophilically digested sludge. After converting the concentrations into kg ton^{-1} TS, the total carbon of mesophilically digested sludge (360 kg ton^{-1} TS) was also higher than that of the thermophilically digested sludge (275 kg ton^{-1} TS). Note that the concentration of tot-N of mesophilically digested sludge provided by Agrilab AB was only half of that shown in Table 7.

Table 8. Analyses of chemical parameters of both substrates done by Agrilab AB

Chemical characteristic:	Mesophilically digested sludge	Thermophilically digested sludge
TS (%)	20	27
Tot-N (kg ton^{-1})	10	7.9
Tot-N (kg ton^{-1} TS)	50	30
Organic N (kg ton^{-1})	8.5	6.1
Organic N (kg ton^{-1} TS)	43	23
NH_4-N (kg ton^{-1})	1.5	1.9
NH_4-N (kg ton^{-1} TS)	7.6	7.0
Tot-C (kg ton^{-1})	71	74
Tot-C (kg ton^{-1} TS)	360	275
C/N	7.2	9.3

5. Discussion

5.1 Mineralization

Mineralization of carbon in sludge, described as the ratio of loss of carbon in its gaseous forms (both CO_2 and CH_4), was more influenced by the substrate than the treatment. Overall, carbon in mesophilically digested sludge was more mineralized than in thermophilically digested sludge (Figure 4 and Figure 6). According to the analysis of chemical properties of sludge measured by Agrilab AB (Table 8), the amount of total carbon in each tube of stored sludge (60 g) at the beginning of the experiment did not differ much between the two types of substrates. However, during the 32-day storage period, around 12.4% (MDR) to 14.9% (MC) of initial total carbon in mesophilically digested sludge was mineralized whereas the mineralized carbon in thermophilically digested sludge ranged from 5.2% (TDR) to 6.5% (TC), see Figure 4. CO_2 emissions from mesophilically digested sludge contributed to about 95.9% (MC) to 98.5% (MDRR) of the total carbon loss while the carbon loss in form of CH_4 was only 1.5% to 4.1%. For thermophilically digested sludge, 93.3% to 97.8% of mineralized carbon was in the form of emitted CO_2 and rest was lost as CH_4 emission (Figure 4 and Figure 6).

According to the results from TS/VS analysis of sludge at the start and at the end of the experiment (Table 4 and Table 5), the reduction in VS content of mesophilically digested sludge during the experiment was much greater than for the thermophilically digested sludge, resulting in a different rate of degradation of organic compounds between substrates. This is consistent with result from previous studies (Harrison-Kirk *et al.*, 2012; Butterly *et al.*, 2010), which focused on the mineralization of carbon in soil. These studies indicated that mineralization was positively related with soil moisture content and also soil organic matter content, which is consistent with the higher mineralization from mesophilically digested sludge where both moisture content and VS content were higher than that of the thermophilically digested sludge.

The difference in mineralization rate between the two types of sludge might also be due to the different C/N ratio of the two substrates. In this experiment, mesophilically digested sludge had a lower initial C/N ratio (7.2) than the thermophilically digested sludge (9.3). Previous studies (Guo *et al.*, 2011; Manzoni *et al.*, 2010) reported that material with a lower initial C/N ratio is considered as a more labile substrate which can enhance the carbon-use efficiency during respiration thus leading to a higher carbon emission, which could explain the higher degradation rate in the mesophilically digested sludge.

Since nitrogen in stored sludge can emit in forms other than N_2O, mineralized N will be underestimated when not taking the losses of N_2, NH_3 and NO during sludge storage into account. Since this study only covered N_2O emissions, N mineralization from stored sludge is not included in this section.

5.2 Effects of drying/wetting on gas production

5.2.1 Carbon dioxide

By comparing the accumulated CO_2 emission from treated and control treatments for both types of sludge during the drying period (Figure 4), it seemed that drying had a slightly negative effect on accumulated CO_2 emission, as also seen in a study by Harrison-Kirk *et al.* (2012). A substantial increase was found in the daily CO_2 emission after applying irrigation for treatments MDR, MDRR, TDR and TDRR. For mesophilically digested sludge, within 2 days after rewetting, emissions from treatment MDR and MDRR reached peaks in CO_2 emission and the emissions increased by 48.7% and 36.0%, respectively, compared with the emissions before irrigation. For thermophilically digested sludge, the CO_2 flux increase in 2 days following irrigation was 33.3% (TDR) and 10.7% (TDRR), though a peak was not observed in the daily CO_2 emission from thermophilically digested sludge since it continued to climb up till the end of the experiments.

As mentioned in the introduction section, drying would gradual increase the air space from the top of the stored sludge thus enabling oxygen diffusion and offering an oxic environment. This facilitated the process of aerobic respiration during which CO_2 was produced. Since aerobic respiration produces more CO_2 compared with anaerobic respiration, CO_2 emitted from the same type of sludge applied with drying ought to be higher than that in the control. This was true for mesophilically digested sludge during the first 9 days of the drying phase. After day 9, however, daily emitted CO_2 from treatment MDR and MDRR remained lower than the emission from MC till the end of the experiment even after rewetting was applied. A possible explanation for this phenomenon for mesophilically digested sludge is that, after 9 days of drying, the moisture content in the top section of stored sludge in MDR and MDRR was too low to sustain the normal rate of aerobic respiration of microbes where enzymes were inhibited by low intracellular water potential, as earlier shown by Stark & Firestone (1995). As a result, CO_2 emission from MC was higher than from the other two treatments during the rest of the drying period. In the beginning of the period after rewetting, the irrigation resulted in recovering moisture content in the top section of the sludge and reactivating aerobiosis, which explains the boost in CO_2 emission after rewetting. Since part of the organic matter in sludge had been degraded during the drying phase, the increase of CO_2 emission after rewetting could hardly compensate for the reduction of CO_2 emitted during the drying phase.

However, daily CO_2 emission from the TC treatment remained higher than the emission from TDR and TDRR over the entire storage phase. One possible explanation for this phenomenon could be that due to its lower moisture content, initial pore space in thermophilically digested sludge was large enough for O_2 to diffuse from the air to the bottom area of the stored sludge, where condition was not as strictly anoxic as expected. Therefore, microbes in the bottom section respired aerobically from the start of the experiment and CO_2 emissions from sludge applied with drying remained lower than that of control treatment, because of the intensive moisture loss in the top section of sludge in TDR and TDRR during the drying phase. Unlike the other substrate, thermophilically digested sludge showed an increasing trend in daily CO_2 production after day 21, which could be the consequence of the activities of mold growing in

the sludge since visible mold growth was first found in tubes of sludge from treatment TDRR on day 22, then was found in all tubes of thermophilically digested sludge on day 23 (Appendix 2).

5.2.2 Methane

Drying had hardly any noticeable impact on CH_4 production during the first week of the drying period. During the following days of the drying period, CH_4 emission stayed at a very low level (less than 0.1 µg CH_4-C g^{-1} tot-C d^{-1}) and negative emissions was registered for all six treatments during the last 2 to 3 days of the drying phase, which might suggest that the bottom section of the stored sludge was actually not anoxic during the first few days of the drying period, since methanogens are oxygen sensitive and can only survive in strict anoxic environment (Madigan *et al.*, 2012).

There was hardly any difference in CH_4 production from treatment TDR before and after sludge irrigation, which was also consistent with treatment TDRR. Daily emitted CH_4 from treatment MDR and MDRR peaked on the third day after rewetting at 0.87 µg CH_4-C g^{-1} tot-C d^{-1} and 50.45 µg CH_4-C g^{-1} tot-C d^{-1}, respectively, with the gas flux before rewetting being -0.03 µg CH_4-C g^{-1} tot-C d^{-1} and 1.72 µg CH_4-C g^{-1} tot-C d^{-1}, respectively (Figure 5). The much greater increase in CH_4 flux from MDRR than MDR was more likely attributed to sludge replacement than rewetting itself since sludge replacement offered new material containing methanogen that was not inhibited by exposure to O_2.

5.2.3 Nitrous oxide

As shown in Figure 7 and Figure 8, during the drying phase, no great difference in neither daily nor accumulated N_2O emission was found between treatments MDR, MDRR and MC. For thermophilically digested sludge, drying led to a relatively lower amount of accumulated emissions from sludge compared with total emitted N_2O from control treatment TC during the drying phase. This could be explained by the hypothesis proposed in section 5.2.1 that at the start of the drying period, the environment in the sludge in both control and treated group was already oxic even in the bottom part where nitrification also took place. Additionally, moisture content of sludge in TC treatment was kept at a relatively high level compared to treatment TDR and TDRR, which enhance the mineralization rate and gas production.

As expected, a great elevation in the daily N_2O emission following rewetting was observed (Figure 7). A 313.2% and 182.1% increase was found in daily N_2O production for treatments MDR and TDRR respectively in the first 24 h after rewetting compared to the emissions before irrigation. It took 2 days for daily N_2O emission from MDRR and TDR to peak after the irrigation and the increase in N_2O flux in treatment MDRR and TDR during this period of time was 215.7% and 187.7%, respectively. Mechanism behind the elevation in N_2O emission could be the reactivation of enzymes in nitrifying bacteria after moisturizing the top section of stored sludge (Stark & Firestone, 1995), and could also be as stated in the hypothesis made in the beginning that the bottom section of stored sludge after rewetting was anoxic which promoted the process of denitrification from which N_2O was produced.

5.3 Effect of substrate on gas production

Substrate played an important role in terms of carbon emission from sludge during the whole storage phase. Carbon released in the form of CO_2 in relation to initial carbon from mesophilically digested sludge (mean of the emissions from three treatments MC, MDR and MDRR) during the entire storage phase was over twice as much as that of thermophilically digested sludge (Figure 4). Difference in CH_4 production between substrates was even higher, with accumulated emissions in relation to initial carbon from mesophilically digested sludge during the entire storage phase being almost seven times higher than the other substrate (Figure 6). C/N ratio could be one of the explanations of this phenomenon since a higher carbon emission from substrate was associated with a lower C/N ratio of the substrate in some previous studies (Guo *et al.*, 2011; Manzoni *et al.*, 2010). Differences in total carbon emission between substrates can also be the consequence of a different extent of mineralization in each substrate caused by variance in moisture content and organic matter (Harrison-Kirk *et al.*, 2012; Butterly *et al.*, 2010). Impact of substrate on N_2O emission from sludge applied with drying and rewetting was relatively low. However, by comparing total emitted N_2O from the two control treatments MC and TC during the whole experiment, thermophilically digested sludge had emitted 30% more N_2O in relation to initial nitrogen than mesophilically digested sludge.

5.4 Nitrogen dynamic in sludge during storage

5.4.1 Mesophilically digested sludge

According to the results from analyses of nitrogen content in sludge (Table 7), NO_3-N was hardly found in any digested sludge at the start of the experiment. After 16 days of drying, NO_3-N concentration in the bottom section of sludge from treatment MDRR increased from 0.1 to 2.4 g NO_3-N kg^{-1} TS while NH_4-N concentration decreased from 6.8 to 3.5 g NH_4-N kg^{-1} TS, proving nitrification at bottom during drying phase.

When comparing NO_3-N and NH_4-N concentration of sludge between top and bottom sections at the end of the experiment, 4.9 and 4.0 g NO_3-N kg^{-1} TS was found in the bottom section of sludge from treatment MDR and MDRR, respectively, where only 0.1 g NH_4-N kg^{-1} TS of ammonium was found. NO_3-N concentration in the top section of sludge from treatments MDR (2.3 g NO_3-N kg^{-1} TS) and MDRR (2.6 g NO_3-N kg^{-1} TS) was lower than that at the bottom, whereas NH_4-N concentration in the top section (2.7 g NH_4-N kg^{-1} TS for treatment MDR and 2.8 g NH_4-N kg^{-1} TS for treatment MDRR) was higher than at the bottom. This phenomenon could probably be due to the inhibition of the nitrifying bacteria's enzyme activity by the low moisture content and intracellular water potential in the top section of the stored sludge (Stark & Firestone, 1995), Compared with the top section, moisture content at the bottom of the sludge from treatment MDR and MDRR was probably more favorable for nitrification, therefore, consumption of NH_4-N took place at the bottom of the sludge and was inhibited in the top section, which is also supported by the higher NO_3-N concentration at the bottom than in the top section.

During the period after the replacement of bottom sludge and the rewetting, an increase in NO_3-N concentration (from 2.4 to 4.0 g NO_3-N kg^{-1} TS) and a reduction in NH_4-N concentration (from 3.5 to 0.1 g NH_4-N kg^{-1} TS) at the bottom of sludge from MDRR was observed. This could be a sign of continuation of nitrification at the bottom section after rewetting. This is also supported by the decreasing concentration of NH_4-N during the period after rewetting. Another explanation of the increase in NO_3^- could be that NO_3^- produced from nitrification at the top section of the stored sludge during drying was presumably percolated with the filtration of irrigation water to the bottom of sludge, according to one of the hypotheses in the introduction chapter, and that only a fraction of the NO_3-N that was accumulated at the bottom experienced anoxic conditions and was denitrified.

At the end of the experiment, the bottom section of sludge from control treatment MC had a NO_3^- concentration of 6.6 g NO_3-N kg^{-1} TS (Table 7) and the NH_4^+ concentration of 4.0 g NH_4-N kg^{-1} TS. Concentration of NO_3^- in the top section of MC, 5.2 g NO_3-N kg^{-1} TS, was lower than the bottom section, while NH_4^+ concentration was higher than the bottom (5.5 NH_4-N kg^{-1} TS). These results indicate that nitrification could take place not only in the top section but also at the bottom of the stored sludge from control treatment. Moreover, during the whole storage phase, a slight reduction was found in TS of sludge sampled from the bottom of MC treatment (Table 4 and Table 5) to which no extra moisture had been ever added during the experiment. One reason for this reduction could probably be that part of the moisture in the upper section of the stored sludge from control treatment MC was drained away due to gravity and was collected at the bottom. Another reason could be the mineralization of organic matter, shown by the emission of CO_2, which decreased the dry matter and released some water. Therefore, it might be possible that NO_3^- produced at the upper section of the stored sludge was transported to the bottom section along the gravity drainage, which might explain the higher NO_3-N concentration at the bottom of sludge from treatment MC.

5.4.2 Thermophilically digested sludge

Similar to mesophilically digested sludge, less than 0.1 g NO_3-N kg^{-1} TS was found in thermophilically digested sludge at the start of the experiment (Table 7). In addition, 6.7 g NH_4-N kg^{-1} TS was found. During the drying period, nitrification took place in the bottom section of the sludge as seen from treatment TDRR since at the bottom concentration increased to 0.4 g NO_3-N kg^{-1} TS while NH_4^+ concentration dropped to 1.6 g NH_4-N kg^{-1} TS. The amount of NO_3-N plus NH_4-N initially was 6.7 g kg^{-1} TS, but after 16 days it was just 2.0 g kg^{-1} TS, in spite of the mineralization that took place during this time. This indicates a considerable denitrification.

Although total emitted N_2O from all six treatments during the whole storage phase did not differ much from each other (Figure 8), NO_3-N concentration in thermophilically digested sludge at the end of the storage phase (0.0-1.6 g NO_3-N kg^{-1} TS) was considerably lower than that of the mesophilically digested sludge (2.3-6.6 g NO_3-N kg^{-1} TS) (Table 7). Unlike mesophilically digested sludge, for which higher NO_3-N concentration was found at the bottom than at the top, NO_3^- concentration at the bottom of sludge from treatment TDR (0.1 g

NO_3-N kg^{-1} TS), TDRR (0.0 g NO_3-N kg^{-1} TS) and TC (1.4 g NO_3-N kg^{-1} TS) was lower than in the top, where it was 0.6, 0.6 and 1.6 g NH_4-N kg^{-1} TS, respectively. Reason for the low NO_3^- concentration in thermophilically digested sludge could probably be the nitrogen assimilation related to mold activities which were particularly found in this type of substrate. The finding of mold growing especially in the lower section of the stored thermophilically digested sludge (Appendix 2), might explain the lower concentrations of NO_3^- in the bottom section than in the top. Besides, at the end of the experiment, neither NO_3^- nor NH_4^+ in the bottom of sludge from TDR and TDRR barely existed (Table 7), which also suggests that inorganic nitrogen might have been assimilated by mold during rewetting.

Moreover, for treatment TDRR, a decrease in NO_3-N concentration at the bottom section during the period after rewetting was found. There was no clear evidence proving if this decrease was the consequence of denitrification after irrigation or nitrogen assimilation, and whether NO_3^- had been percolated from top to bottom during the event of irrigation was also unclear. Therefore, the mechanism behind the nitrate reduction during rewetting phase in this case is hard to understand.

5.5 Limitations and further studies

Even though the experiment was carefully designed and tested before it started, limitations and shortcomings still existed.

First of all, frequency of ventilations and gas sampling scheduled for treated group and controls were different. Gas sampling for treatment with drying and rewetting was scheduled on daily basis during the first week of both initial drying and after rewetting. The reason for this was that we aimed at obtaining an accurate image of the trend of the gas production on each day during this period since first week of each phase was usually the time when gas emissions varied much. Besides, if those units were not sampled daily, the gas concentration would be at risk to become too high and thereby exceed the detection limit of the GC. However, since we were limited in not only the time for gas sampling and preparation work for gas analyses but also the cost in running GC for gas analysis, and since the gas concentration expected in the control group was not as high as in the other treatments the concentration in gas samples taken from control group would still stay within the detection range even if they were less ventilated. Moreover, ventilation not preceded with a gas sampling would lead to an absence of the value of the emission for that day, which would mean that the calculation of accumulated gas production would be less accurate. As a result, gas produced from control treatments was sampled less frequently than the treated group.

The different sampling frequency brought difficulties when analyzing the gas production in this study. For example, an unexpected fluctuation was found in daily CO_2 emission from treatment MC (Figure 3), and reason for this instability might be the difference in time between each gas sampling occasion. When there was a less frequent sampling (once in three days), there was a reduction in daily emission. When gas from the control treatment was sampled every other day, the daily gas emission increased again. Therefore, different

sampling frequencies among different treatments might result in altering the dynamics of gas flux thus making gas analysis more uncertain.

Secondly, difficulties also lied in accurately simulating the natural storage condition of sewage sludge by experiment at lab scale. In order to well represent the gradual top-to-bottom evaporation of moisture in stored sludge and ensure an anoxic environment in the bottom section similar to the condition of natural stored sludge, a narrow and relatively long tube (42 mm in diameter, 144 mm in length) was selected as container for storing the sludge in the experiment. However, at the end of the drying period, the stored sludge applied with drying and rewetting had shrunk horizontally thus forming a conical shape creating a slot between tube and sludge (Appendix 2) through which air could easily diffuse into the lower section of stored sludge where condition was no longer strictly anoxic, which was proved by the occurrence of nitrification in the bottom of stored sludge and absence of methanogesis during the second half of the experiment. Therefore, stored sludge in this experiment could probably only represent the upper layer, or perhaps even only the very top layer, of stored sludge in reality.

When analyzing chemical properties of sludge samples from each sludge sampling, a series of sludge solutions with dilutions of 1/5, 1/50, 1/500 for sludge sample was done to achieve the desired chemical concentration which could fit in the measuring range for each chemical measurement. Sludge samples that was paste-like or had dry matter content around or below 30% could be easily diluted as a roughly homogeneous aqueous suspension. However, for sludge samples which had higher TS (e.g. sludge sampled from top section of stored sludge), it was difficult to achieve a homogeneous suspension for the 1/5 dilution of sludge, since the texture of the dried sludge was hard. Therefore, when drawing original solution (1/5 dilution) for further diluting, accuracy of the chemical concentration in the following serial solutions of 1/50 and 1/500 dilution, which should be diluted by a factor of 10 and 100, respectively, compared with original solution, was negatively affected. Moreover, as total nitrogen is only partly soluble in water, sludge solution (which in this case is always a 1/500 dilution) has to be digested before determining the concentration of total nitrogen. As the uncertainty in the dilution process was mainly on the insoluble compounds, it was even more unsure whether the total nitrogen present in the 1/500 serial dilution well represented the concentration in the sludge. Therefore, the uncertainty in the total-N concentration values reported for sludge samples can be predicted to be large. This is also seen by the large difference in the values given in Table 7 measured with the method described above and the values measured by Agrilab (Table 8). The uncertainty in the analyses of the total nitrogen is the reason for the absence of interpretation of total nitrogen development during sludge storage in the discussion section.

Sludge which has been subjected to thermophilic digestion is expected to have less volatile fatty acids than sludge that has been subjected to mesophilic digestion of same period of short time (de la Rubia *et al.*, 2002; Nosrati *et al.*, 2011) and may result in different organic matter content which is an important factor affecting mineralization (Harrison-Kirk *et al.*, 2012) and thus gas emission. However, the sludge subjected to thermophilic and mesophilic digestion

respectively was collected from different municipalities and was processed in different treatment plants. Besides, due to the insufficient dewatering caused by the replacement of the centrifuge in Uppsala Vatten, the sewage sludge produced from the two treatment plants also differed in moisture content which might have an impact on microbial activity in sludge and consequently affect the gas emission as well. Based on those factors, it will be inaccurate to draw the conclusion that applying a different digestion temperature during sewage treatment for certain has an impact on gas emission during the storage.

Further study is advised to focus on investigating the spatial and temporal variability on the physical and chemical properties of stored sludge (moisture, pH, oxygen content, substrate availability), so as for laboratory study to better simulating natural condition of stored sludge. Furthermore, since low pH was found in the stored sludge, which might change the portion of products of denitrification and affect the dynamic of nitrogen, research on emission of NO, another by-product from both nitrification and denitrification, from sludge during storage is suggested. It would also be of interest to study both thermophilic and mesophilic digestion for the same substrate.

6. Conclusions

Compared with accumulated emission from sludge stored at constant moisture, drying during storage of sludge slightly reduced the accumulated emission of CO_2 from both thermophilically and mesophilically digested sludge and also reduced the emission of N_2O from the thermophilically digested sludge. No great difference was found in accumulated N_2O production between MDR/MDRR and MC during drying.

A substantial increase in the daily emission of CO_2 from MDR and MDRR after rewetting was observed. Rewetting also led to a considerable increase in daily emission of N_2O from both the thermophilically and the mesophilically digested sludge. However, drying and rewetting hardly made any difference on CH_4 emission.

There was a big difference in the carbon emission between the thermophilically and the mesophilically digested sludge during the whole storage phase. Carbon released in the forms of CO_2 and CH_4 during the entire storage phase was over twice and seven times as much from mesophilically digested sludge as from thermophilically digested sludge, respectively. Impact of substrate on N_2O emission in relation to initial total nitrogen from sludge applied with drying and rewetting was relatively low, however, by comparing total emitted N_2O from two control treatments, MC and TC, thermophilically digested sludge emitted 30% more N_2O than the other substrate.

References

Barros, N., Gomez-Orellana, I., Feijóo, S., & Balsa, R. (1995). The effect of soil moisture on soil microbial activity studied by microcalorimetry. *Thermochimica Acta, 249*(0), 161-168.

Beare, M. H., Gregorich, E. G., & St-Georges, P. (2009). Compaction effects on CO_2 and N_2O production during drying and rewetting of soil. *Soil Biology and Biochemistry, 41*(3), 611-621.

Bitton, G. (2011). *Wastewater microbiology* (4th ed.). Hoboken, N.J.: Wiley-Blackwell.

Bremner, J. M., & Shaw, K. (1958). Denitrification in soil. II. Factors affecting denitrification. The Journal of Agricultural Science, 51, pp 40-52.

Butterly, C. R., Marschner, P., McNeill, A. M., & Baldock, J. A. (2010). Rewetting CO_2 pulses in Australian agricultural soils and the influence of soil properties. *Biology and Fertility of Soils, 46*(7), 739-753.

Cayuela, M. L., Sánchez-Monedero, M. A., Roig, A., Hanley, K., Enders, A., & Lehmann, J. (2013). Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions? *Scientific Reports, 3*

Davidson, E. A., Verchot, L. V., Cattânio, J. H., Ackerman, I. L., & Carvalho, J. E. M. (2000). Effects of Soil Water Content on Soil Respiration in Forests and Cattle Pastures of Eastern Amazonia. *Biogeochemistry, 48*(1), 53-69.

de la Rubia, M.A., Perez, M., Romero, L.I. & Sales, D. (2002). Anaerobic mesophilic and thermophilic municipal sludge digestion. *Chemical and Biochemical Engineering Quarterly*, 16 (3), 119-124.

Doran, J. W., Mielke, L. N., & Power, J. F. (1990). Microbial activity as regulated by soil water-filled pore space. *Transactions 14th International Congress of Soil Science, Kyoto, Japan.* Volume III. 1990 pp. 94-99, Lincoln: University of Nebraska–Lincoln.

Flodman, M. (2002). Emissioner av metan, lustgas och ammoniak vid lagring av avvattnat rötslam (Emissions of methane, nitrous oxide and ammonia during storage of dewatered sludge). *Meddelande 2002:4, Institutionen för lantbruksteknik. Sveriges Lantbruksuniversitet, Uppsala, Sverige.*

Goldberg, S. D., & Gebauer, G. (2009). Drought turns a Central European Norway spruce forest soil from an N_2O source to a transient N_2O sink. *Global Change Biology, 15*(4), 850-860.

Groffman, P. M., Altabet, M. A., Bohlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., Kana, T. M., Nielsen, L. P., & Voytek, M. A. (2006). Methods for

measuring denitrification: diverse approaches to a difficult problem. *Ecological Applications, 16*(6), 2091-2122.

Gubry-Rangin, C., Nicol, G. W. & Prosser, J. I. (2010). Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS Microbiology Ecology*, 74: 566–574.

Guo, R., Li, G., Jiang, T., Schuchardt, F., Chen, T., Zhao, Y., & Shen, Y. (2012). Effect of aeration rate, C/N ratio and moisture content on the stability and maturity of compost. *Bioresource Technology, 112*, 171-178.

Harrison-Kirk, T., Beare, M. H., Meenken, E. D., & Condron, L. M. (2013). Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions. *Soil Biology and Biochemistry, 57*(0), 43-55.

Heincke, M., & Kaupenjohann, M. (1999). Effects of soil solution on the dynamics of N_2O emissions: a review. *Nutrient Cycling in Agroecosystems, 55*(2), 133-157.

HORIZONTAL. (2007). Standards for inorganic parameters with performance characteristics. *ECN*. Retrieved on September 23, 2013, from http://horizontal.ecn.nl/final-documents-including-validation-data/inorganic-parameters/

IPCC (Intergovernmental Panel on Climate Change). (2007). Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., & Miller, H.L., ed., *Climate Change 2007: The Physical Science Basis*, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, ISBN 978-0-521-88009-1

Kato, M. T., Field, J. A., & Lettinga, G. (1993). High tolerance of methanogens in granular sludge to oxygen. *Biotechnology and Bioengineering, 42*(11), 1360-1366.

Kessavalou, A., Doran, J. E., Mosier, A. R., & Drijber, R. A. (1998). Greenhouse gas fluxes folowing tillage and wetting in a wheat-fallow cropping system. *Journal of Environmental Quality, 27*.

Kim, D. G., Vargas, R., Bond-Lamberty, B., & Turetsky, M. R. (2012). Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research. *Biogeosciences, 9*(7), 2459-2483.

Knowles, R. (1982). Denitrification. *Microbiol Reviews, 46*(1), 43-70.

Konneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., & Stahl, D. A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. *Nature, 437*(7058), 543-546.

Kosobucki, P., Chmarzyński, A., & Buszewski, B. (2000). Sewage Sludge Composting. *Polish Journal of Environmental Studies, 9*(4), 243-248.

Linn, D. M., & Doran, J. W. (1984). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. *Soil Science Society of America Journal, 48*(6), 1267-1272.

Lorenz, K., & Lal, R. (2010). *Carbon sequestration in forest ecosystems*. Dordrecht: Springer.

Machefert, S. E., & Dise, N. B. (1999). Hydrological controls on denitrification in riparian ecosystems. *Hydrology and Earth System Sciences, 8*(4), 686-694.

Madigan, M. T., Martinko, J. M., Parker, J., & Brock, T. D. (2012). *Brock biology of microorganisms* (13th ed.). San Francisco: Benjamin Cummings.

Manzoni, S., Trofymow, J. A., Jackson, R. B., & Porporato, A. (2010). Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecological Monographs, 80*(1), 89-106.

Nosrati, M., Amani, T. & Sreekrishnan, T. R. (2011). *Thermophilic Anaerobic Digestion of Waste Activated Sludge versus Mesophilic Anaerobic Digestion.* Paper presented at the International Conference on Advances in Biotechnology and Pharmaceutical Sciences (ICABPS'2011). Bangkok.

Papendick, R. I., & Camprell, G. S. (1981). Theory and measurement of water potential. Parr, J.F., Gardner, W.R., & Elliott, L.F. (ed.) *Water Potential Relations in Soil Microbiology, Soil Science Society of America,* 1–22.

Paredes, C., Roig, A., Bernal, M. P., Sánchez-Monedero, M. A., & Cegarra, J. (2000). Evolution of organic matter and nitrogen during co-composting of olive mill wastewater with solid organic wastes. *Biology and Fertility of Soils, 32*(3), 222-227.

Princic, A., Mahne, I. I., Megusar, F., Paul, E. A., & Tiedje, J. M. (1998). Effects of pH and oxygen and ammonium concentrations on the community structure of nitrifying bacteria from wastewater. *Applied and Environmental Microbiology, 64*(10), 3584-3590.

Samarkin, V. A., Madigan, M. T., Bowles, M. W., Casciotti, K. L., Priscu, J. C., McKay, C. P., & Joye, S. B. (2010). Abiotic nitrous oxide emission from the hypersaline Don Juan Pond in Antarctica. *Nature Geoscience, 3*(5), 341-344.

Skiba, U., Smith, K. A., & Fowler, D. (1993). Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. *Soil Biology and Biochemistry, 25*(11), 1527-1536.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., & Smith, J. (2008). Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*(1492), 789-813.

Stark, J. M., & Firestone, M. K. (1995). Mechanisms for soil moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology, 61*(1), 218-221.

Syamsul Arif, M. A., Houwen, F., & Verstraete, W. (1996). Agricultural factors affecting methane oxidation in arable soil. *Biology and Fertility of Soils, 21*(1-2), 95-102.

Tarre, S., & Green, M. (2004). High-rate nitrification at low pH in suspended - and attached - biomass reactors. *Applied and Environmental Microbiology, 70*(11), 6481-6487.

Tenenbaum, D. (1997). The Beauty of Biosolids. *Environmental Health Perspectives, 105*(1), 32-36.

White, R. E. (2005). Principles and Practice of Soil Science: The Soil as a Natural Resource (4^{th} ed.). Malden, MA: Blackwell.

Wrage, N., Velthof, G. L., van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry, 33*(12–13), 1723-1732

Xu, X., & Luo, X. (2012). Effect of wetting intensity on soil GHG fluxes and microbial biomass under a temperate forest floor during dry season. *Geoderma, 170*(0), 118-126.

Personal communication

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Appendix 1 - Nitrogen content and pH of sludge samples at day 0, 16 and 32 of the experiment (n=3)

Sampling date, location and treatment			Mesophilically digested sludge				Thermophilically digested sludge			
			NH_4-N	NO_3-N	Tot-N	pH	NH_4-N	NO_3-N	Tot-N	pH
			g kg^{-1} TS	g kg^{-1} TS	g kg^{-1} TS		g kg^{-1} TS	g kg^{-1} TS	g kg^{-1} TS	
Day 0		Mean	6.8	0.1	90	7.46	6.7	0.0 [1)]	36	7.54
		SE	0.1	0.0	6	0.01	0.0	0.0	2	0.03
Day 16, bottom	DRR	Mean	3.5	2.4	52	--	1.6	0.4	49	--
		SE	0.1	0.1	5	--	0.2	0.0	7	--
Day 32, top	DR	Mean	2.7	2.3	23	6.42	2.2	0.6	20	6.41
		SE	0.1	0.1	2	0.06	0.2	0.0	6	0.02
	DRR	Mean	2.8	2.6	17	6.32	2.1	0.6	9	6.42
		SE	0.2	0.1	3	0.04	0.2	0.1	1	0.05
	C	Mean	5.5	5.2	52	6.12	1.3	1.6	40	6.10
		SE	0.4	0.1	2	0.05	0.2	0.2	5	0.03
Day 32, bottom	DR	Mean	0.1	4.9	47	4.84	0.0 [2)]	0.1	27	6.24
		SE	0.1	0.3	4	0.10	0.0	0.0	1	0.02
	DRR	Mean	0.1	4.0	55	5.59	0.0 [3)]	0.0 [4)]	30	6.36
		SE	0.0	0.4	8	0.13	0.0	0.0	1	0.03
	C	Mean	4.0	6.6	59	5.49	0.2	1.4	28	5.95
		SE	0.5	0.3	1	0.08	0.1	0.2	1	0.03

1) 0.045 g kg^{-1} TS; 2) 0.011 g kg^{-1} TS; 3) 0.006 g kg^{-1} TS; 4) 0.015 g kg^{-1} TS, Note that no pH measurement was done for sludge sampled on day 16.

Appendix 2 - Stored sludge at the end of the experiment

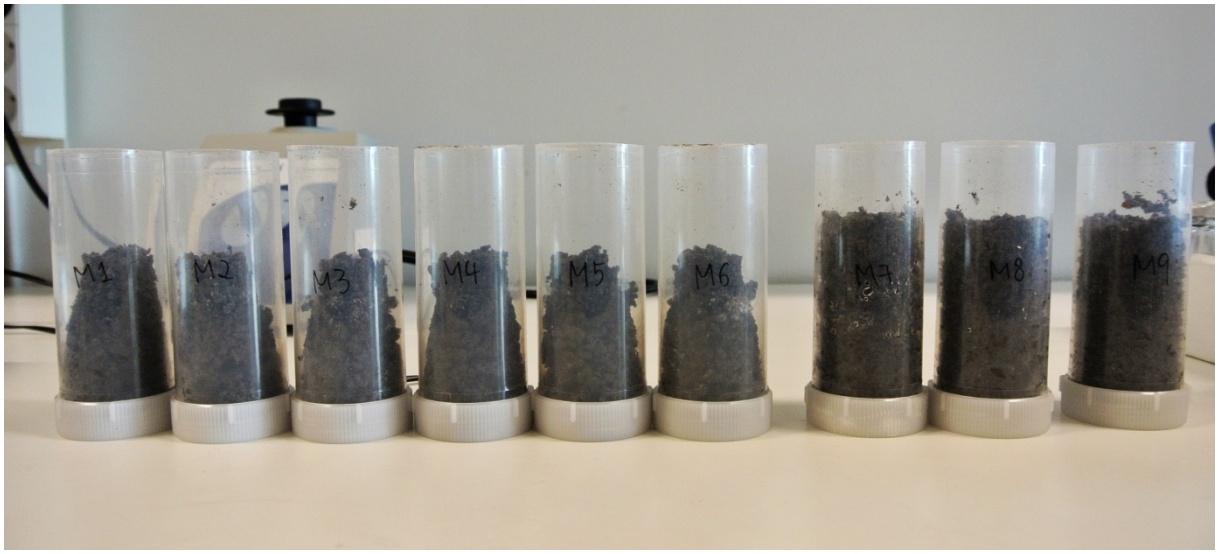

Figure A2:1. Tubes filled with mesophilically digested sludge. ID of tubes from left to right: M1, M2 and M3, belongs to treatment MDR; M4, M5 and M6, belongs to treatment MDRR; M7, M8 and M9, belongs to treatment MC. Personal photograph by Xi Yang. 17 May 2013.

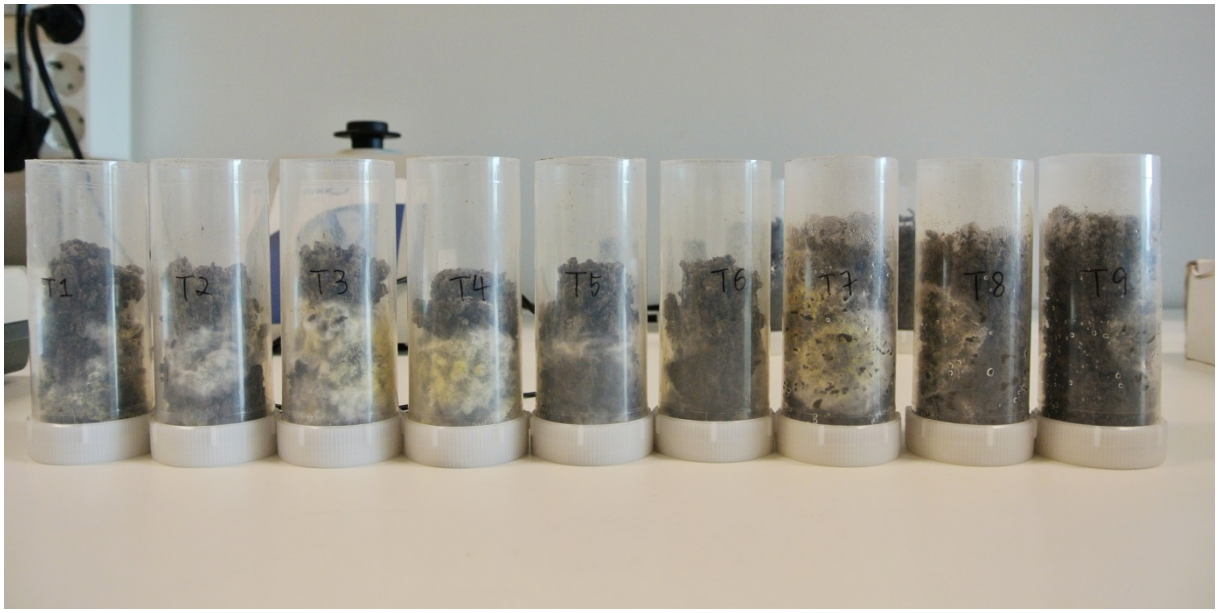

Figure A2:2. Tubes filled with thermophilically digested sludge. ID of tubes from left to right: T1, T2 and T3, belongs to treatment TDR; T4, T5 and T6, belongs to treatment TDRR; T7, T8 and T9, belongs to treatment TC. Personal photograph by Xi Yang. 17 May 2013.

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